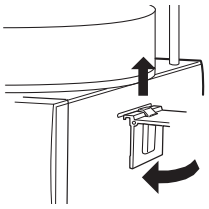
8.2 Optical unit

8.2.1 Connecting the optical unit holder

Hook the holder into the slot on the right hand side of the system. Secure it by pushing up the slide clamp.



8.2.2 Changing UV flow cell

A preparative (2 mm) flow cell is included in the system. An analytical (5 mm) flow cell is available as an accessory. The flow cell can be changed when required, for example from 2 mm to 5 mm to increase the sensitivity, or from 5 mm to 2 mm to decrease the sensitivity.

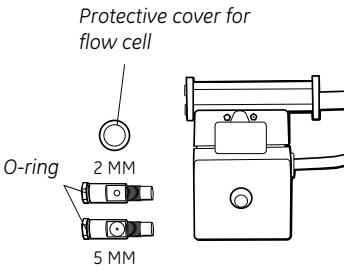
Change the flow cell as follows:

1 Disconnect the inlet and the outlet capillaries from the flow cell.

2 Loosen the flow cell by turning the locking nut and remove it.

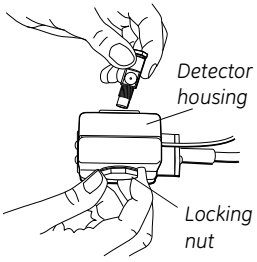
3 Remove the protective cover from the old flow cell and transfer it to the new flow cell.

4 Insert the new flow cell into the detector housing from above.



Note: *The flow cell can only be placed in one correct position.*

5 Secure the flow cell by turning the locking nut until it reaches its stop position.



Note: *If the locking nut is not tightened sufficiently, the monitor will function poorly (e.g. drifting base-line).*

6 Place the protective cover around the flow cell to protect the electronics inside the optical unit from liquid spillage.

Note: *Ensure that the Hg lamp position and the filter are selected according to the wavelength to be used. This is described in the 8.2.5 Changing the lamp assembly (optional) section below.*

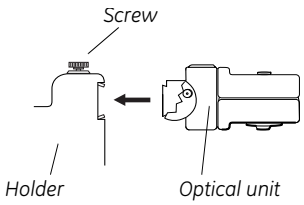
8.2.3 Connecting the optical unit to the system

If the optical unit has been disconnected from the system, connect it as follows:

1 Place the optical unit in the holder.

2 Secure it by tightening the screw in the holder.

3 Connect the lamp cable to the socket **UV Lamp** on the rear panel of the module.



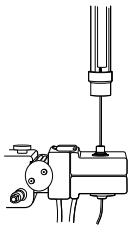
4 Connect the signal cable to the socket **UV** on the rear panel of the module.

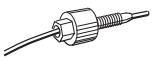
8.2.4 Connection to the column

1 Fix the optical unit directly under the column if possible.

Note: *Always position the optical unit with the filter wheel cover facing upwards.*

2 Connect the column outlet tubing directly onto the top of the optical unit using a fingertight connector and screw to finger-tightness.





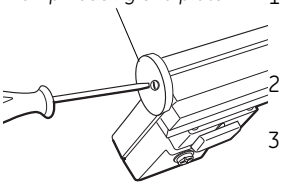
3 Connect the optical unit outlet tubing onto the opposite hole in the flow cell. Use fingertight connectors.

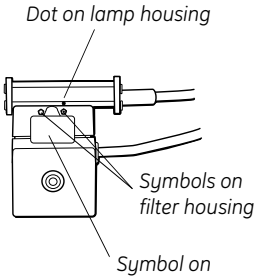
If no outlet tubing exists, cut a piece of PEEK tubing (i.d. 0.75 mm, o.d. 1/16"). The length should be 170 mm.

4 Connect the other end of the tubing to the conductivity flow cell.

8.2.5 Changing the lamp assembly (optional)

WARNING! The system uses high intensity ultra-violet light. Do not remove the UV lamp while the system is running. Before replacing a UV lamp, ensure that the lamp cable is disconnected from the rear of the system to prevent injury to the eyes. If the mercury lamp is broken, make sure that all mercury is removed and disposed according to national and local environmental regulations.





1 Use a screwdriver to detach the end plate by removing one and loosening the other of the two holding screws on the lamp housing to be removed.

2 Slide the lamp housing off the filter housing.

3 Detach the end plate, as in step 1 above, from the lamp housing to be fitted to the optical unit.

4 Slide the lamp housing onto the filter housing. The lamp and signal cables should be on the same side. As you slide the lamp housing into position, depress the two pressure pads on the filter housing in sequence to facilitate the installation.

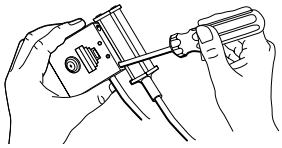
5 Refit the lamp housing end plate.

6 Slide the lamp housing firmly into place. There will be a faint click when the housing is positioned correctly. The Hg lamp housing can take up two positions, one for 280 nm, marked by ○ on the filter housing, and the other marked by for ● all other wavelengths. The Zn lamp housing has only one position.

7 Set the wavelength to be used by selecting lamp position (indicated by a dot on the lamp housing) in combination with the appropriate filter, i.e. the dot on the lamp housing should be adjacent to the symbol on the filter housing corresponding to the symbol on the filter wheel for the filter to be used. A click will indicate that the filter is in position.

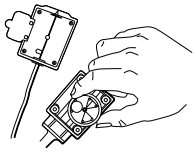
8.2.6 Changing the filter (optional)

The Hg optics with 254 and 280 nm filters and the Zn optics with the 214 nm filter are delivered with filters installed. If other filters are to be used, install them as follows:



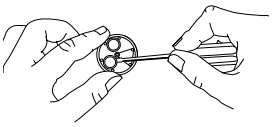
1 If the Zn lamp is attached, remove the lamp housing as described in section 8.2.5 Changing the lamp assembly (optional).

2 Remove the four screws in the filter housing. Separate the filter housing from the detector housing.

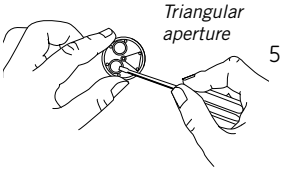


3 Carefully remove the filter wheel from the filter housing.

4 If necessary, remove the filter(s) from the filter wheel by pressing it (them) out, e.g. with a small screwdriver.



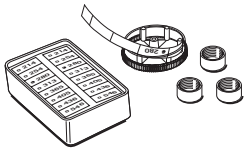
Note: *Filters are sensitive optical components. Never touch the optical surfaces or expose them to temperatures above 60 °C. Clean them with dry lens cleaning tissue and store them, when not in use, in the box in which they were supplied. Heavy contamination may be removed by using a lens tissue dipped in ethanol.*



5 Insert the filter(s) of choice into the filter wheel (maximum 3 filters) with the correct orientation (the mirror side facing upwards) and position over one of the three triangular apertures. The filters snap in by pressing them quite firmly. Do not touch the filter surface.

6 Remove the circular plastic band showing the wavelength(s).

7 Remove labels from the band if necessary.



8 Place the correct labels on the band with the label designation facing outwards. Ensure that the label position corresponds to the filter position, i.e. the label should be placed opposite the filter.

9 Reassemble the circular plastic band with the filter wheel peg fitting into the band notch.

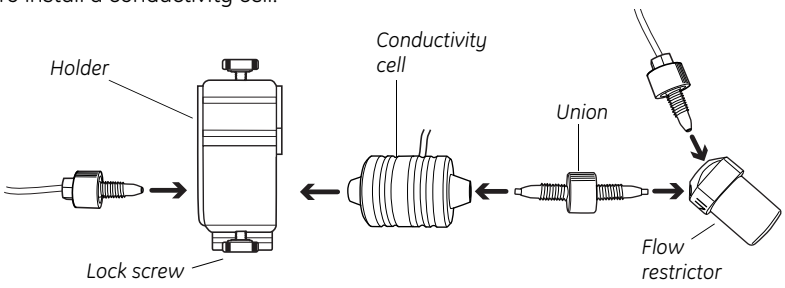
10 Check that all filters are clean. Insert the filter wheel back into the filter housing.

Note: *The filter wheel can be placed only in one correct position.*

11 Reassemble the filter housing with the detector housing by fastening the four screws.

8.3 Conductivity cell

To install a conductivity cell:

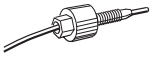


1 Connect the conductivity cell to the flow restrictor using the union.

2 Insert the cell into the holder and secure it with the lock screw.

Note: *When the conductivity flow cell is used with the pH electrode, place the conductivity flow cell and select its flow direction so that the screw head end of the flow cell faces the flow restrictor.*

3 Connect the conductivity cell to the socket **Conductivity Flow Cell** on the rear panel of the system.



4 Connect the tubing with fingertight connectors.

8.4 Fraction collector

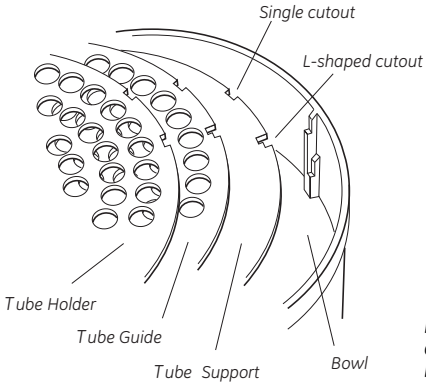
8.4.1 Assembling the tube rack

There are three types of tube racks:

Rack	Max. no. tubes	Tube diam.	Tube length
12 mm	175	12 mm	50-180 mm
18 mm	95	10-18 mm	50-180 mm

ÄKTAprime plus is delivered with the 18 mm rack mounted. Each rack is made up of a combination of a bowl, tube supports, tube guide and tube holder.

The 12 mm rack is available as accessory. Also available for use with the 12 mm tube rack is a double-ended Eppendorf tube holder (18-8522-01). One end holds 1.5 ml Eppendorf tubes, the other holds 0.5 ml tubes.



Note that the tube guide has both single and L-shaped cutouts while the tube holder only has single cutouts.

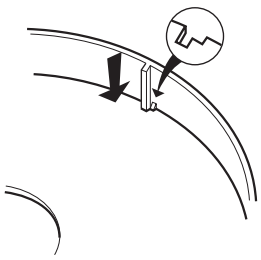
When assembling a rack, different cutouts are used for the various items depending on the length of the tubes. The cutouts are summarized in the tables below.

Tube racks, 12 and 18 mm

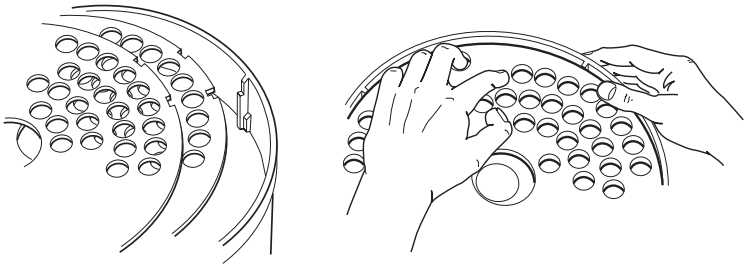
Item	50-85 mm tubes	85-100 mm tubes
Tube support	L-shaped cutout	Not required
Tube guide	Single cutout	L-shaped cutout
Tube holder	Single cutout	Single cutout

1 Insert the tube support, if required, in the bowl. The circular marks on the support should face down.

2 Insert the tube guide with the tube numbers facing upwards. The guide should rest about 1 cm above the support.



3 Insert the tube holder, checking that position 1 is directly above position 1 in the tube guide. Push the flexible bowl out at each rib and snap the holder under the top of the rib.

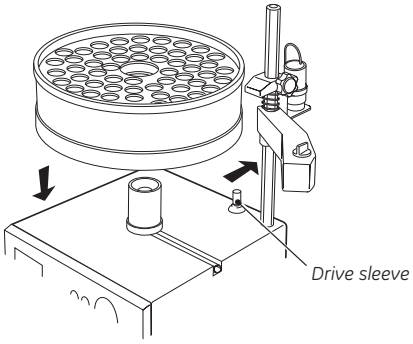


Do not force the holder into place as this may damage the lip. The surface of the holder should be level.

8.4.2 Mounting the tube rack

1 Gently move the delivery arm out to the second stop.

2 Place the rack over the central spindle and pull the spring loaded drive sleeve out so that the rack comes to rest.

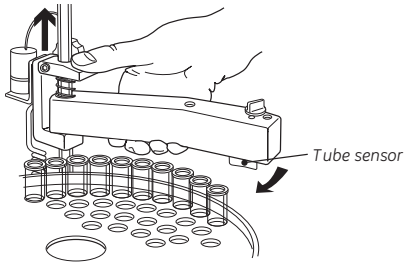


8.4.3 Inserting the collection tubes

Insert the sufficient collection tubes into the rack, starting in position 1, pushing each one down as far as it will go. All the tubes must be of the same length and diameter, and there should be no empty spaces in the sequence.

8.4.4 Adjusting the delivery arm

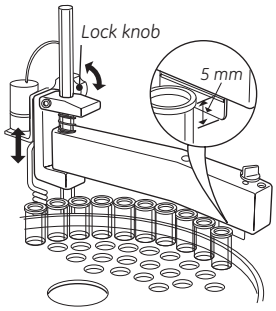
1 Lower the arm and allow it to move so that the tube sensor touches the collection tubes of the outer track.

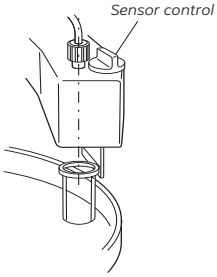


2 Adjust the arm bracket so that the bottom of the tube sensor is about 5 mm below the top of the tubes. The tubes should always be below the horizontal mark on the tube sensor

3 Lock the arm bracket at this height with the lock knob.

4 Rotate the rack counter clockwise by hand, until the rear half of the tube sensor rests against tube 1. When the fraction collector is started, the bowl moves to the correct position to collect the first fraction in tube 1.





5 Check that the sensor is in the correct position for the tube size. The eluent tubing should be over the centre of the collection tube. Use the red sensor control to position the tube holder.

8.4.5 Connecting tubing

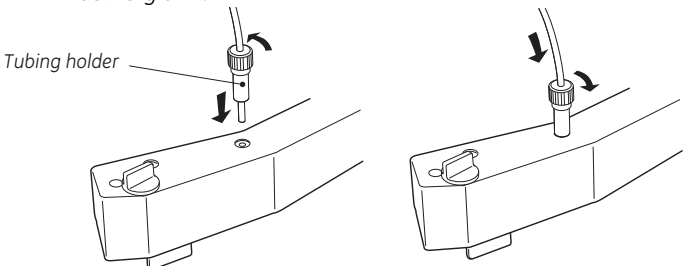
Select the tubing with the required inner diameter 0.75 mm. To change the tubing, follow steps 1–5.

WARNING! When using hazardous chemicals, avoid spillage during fraction collection and when the delivery arm is moved out.

1 Fit a 41 cm long tubing by lifting out the tubing holder from the delivery arm, loosening the nut and then inserting the tubing.

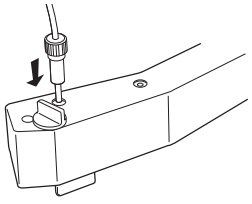
Note: *Check that the tubing end is cut leaving a straight edge.*

Note: *The tubing must be long enough to ensure free movement of the delivery arm.*



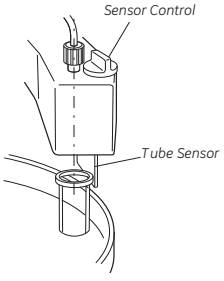
2 Place the tubing holder over the length guide (small hole) in the delivery arm, push the tubing down to the bottom of the guide and tighten the nut. This ensures that the correct length of tubing is exposed.

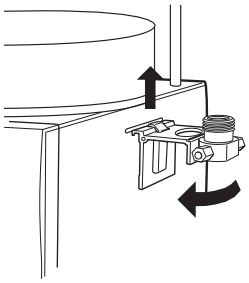
3 Re-install the tubing holder into the delivery arm.



4 Set the red sensor control to position the tubing over the centre of the collection tube.

5 Connect the other end of the tubing to port NC of the flow diversion valve.





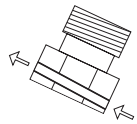
8.5 pH flow cell and electrode (optional)

8.5.1 Mounting the flow cell holder

In the ÄKTAprime plus system, the pH electrode is optional.

1 Hook the flow cell holder on the right hand side of the housing. Secure it with the slide clamp.

If the flow cell holder is not used, the flow cell must still be installed at an angle of 30° from the vertical with the outlet placed higher than the inlet to prevent air bubbles being trapped in the cell.
The flow direction is marked on the flow cell.



Flow direction

2 Connect the tubing with finger-tight connectors.

8.5.2 Inserting the pH electrode

Note: *Handle the pH electrode with care.*

CAUTION! The tip of the pH electrode consists of a thin glass membrane. Protect it from breakage, contamination and drying out or the electrode will be destroyed. Always store the electrode with the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3. Do NOT store in water only.

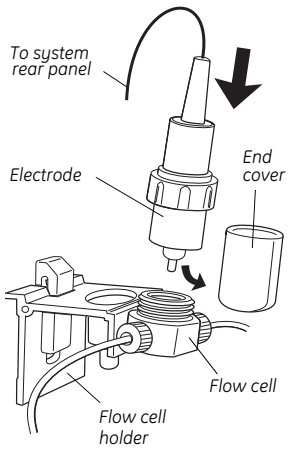
1 Unpack the pH electrode. Ensure that it is not broken or dry.

2 Before using the electrode, remove the electrode end cover and immerse the glass bulb in buffer for 30 minutes.

3 Carefully insert the electrode in the flow cell. Tighten the nut by hand.

Note: *If the flow cell is full of liquid, it is not possible to insert the electrode. If so, loosen the inlet connection while inserting the electrode to allow the liquid to run out from the flow cell. Remember to re-tighten the connector.*

Note: *If the electrode is not fully inserted, the system will leak and a dead volume will occur in the holder.*



4 Connect the pH electrode cable to the socket **pH-Probe** on the rear of the system.

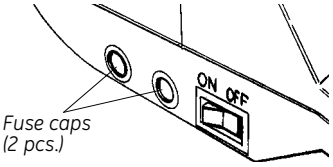
8.6 Recorder REC 112

ÄKTAprime plus can be delivered with a recorder, REC 112, for simpler data presentation. This section describes have to install and use the recorder.

8.6.1 Electrical connections

1 Make sure that the mains power switch is in the **OFF** position.

2 Insert the correct fuses into the fuse caps.



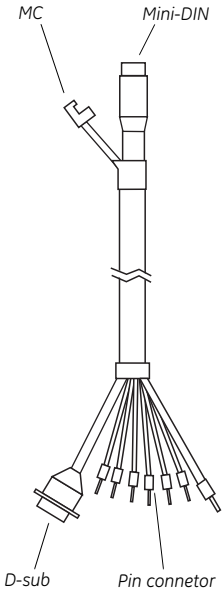
Fuse caps (2 pcs.)

- For 100–120 V operation, use the 250 mA fuses supplied.
- For 220–240 V operation, use the 125 mA fuses supplied.

3 Fit the fuse caps into the fuse holder sockets on the left side of the recorder.

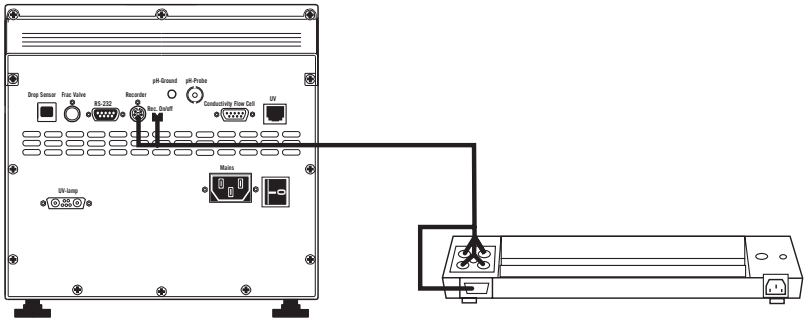
4 Make sure that the mains voltage selector at the rear of the recorder is set to the mains voltage of the laboratory.

CAUTION! The mains voltage selector must be set to the mains voltage of the laboratory. If not, electronics might be damaged.

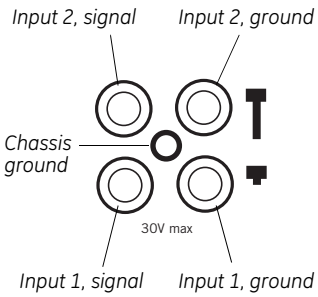


5 Connect the supplied signal cable to ÄKTAprime plus as follows:

- The mini-DIN connector to socket **Recorder**.
- The MC connector to socket **Rec On/off.**
  The left-hand pin in the socket is the ON/OFF-signal and the right-hand pin is signal ground.



6 Connect the pin connectors at the other end of the cable to the signal input plugs supplied with the recorder.



Note: *The signal cable is delivered with protective covers on each wire. Do not remove the protective covers from unused connections as this may disturb the measurement.*

7 Connect the plugs to the signal inputs on the recorder.
Pin designations for the signals (and colors on the corresponding cable wire) are as follows:

Pin no.	*Signal*[1]	Range
1 (Brown)	Signal 1	0–1 V
2 (Red)	Signal 1 ground	0 V
3 (Orange)	Signal 2	0–1 V
4 (Yellow)	Signal 2 ground	0 V
5 (Green)	Signal 3	0–1 V
6 (Black, thin)	Signal 3 ground	0 V
7 (Black, thick)	Chassis ground	0 V

[1] The signals have the following default parameter settings:
Signal 1 – UV absorbance
Signal 2 – %B
Signal 3 – Conductivity
These settings can be changed (see section 8.6.5 Setting analog outputs).

8 Connect the D-sub connector to the D-sub socket in the recorder. Pin 12 is the ON/OFF-signal and pin 15 is signal ground.

9 Connect the mains cable to a properly grounded mains socket.

WARNING! The system must be connected to a grounded mains socket to prevent system parts from becoming live.

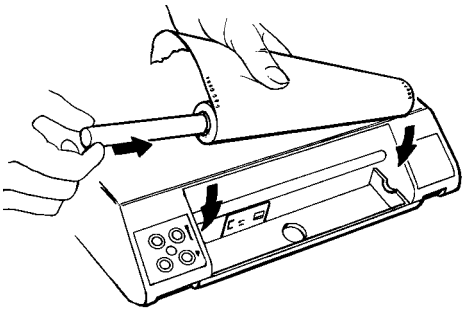
10 Turn on the mains power to the recorder.

8.6.2 Loading the chart paper roll

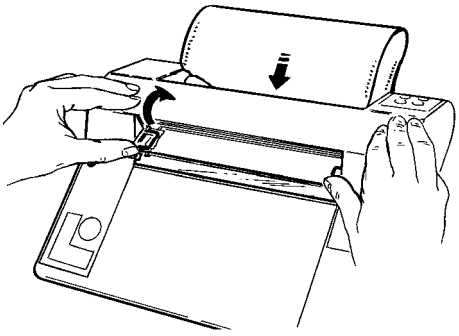
To load the chart paper roll:

1 Unpack the paper roll supplied with the recorder and put into place (1.). Make sure the paper roll is positioned so that the printed grid faces the user during normal operation.

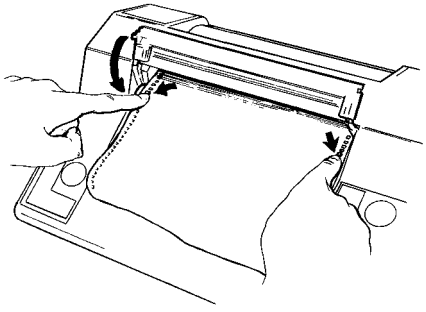
2 Lift the plastic transparent ruler (2.) by pushing it gently upwards so that it rests over the top of the recorder. Leave it in this position.



1.



2.



3.

3 Pull the paper forward. It should be parallel to the housing.

4 Use your fingertips to fit the holes in the edge of the paper over the sprockets. Do this on the right-hand side first and then on the lefthand side (3.). This is important because the holes in the right side guarantee exact paper positioning whereas the holes on the left side only support transport.

5 When the paper is correctly positioned over both sprocket wheels, hold the paper in position and lower the ruler.

6 Use both hands to gently apply pressure to both the left and right edge of the ruler. A double click indicates that the ruler is properly positioned.

8.6.3 Installing the pens

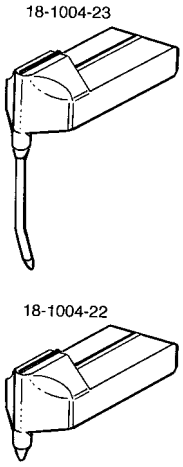
Recorder REC 112 has a writing system that is very simple and convenient. It uses reliable fibre-tip pens of the disposable cartridge type (1.), which leave a very fine trace and do not bleed.

To install the pens:

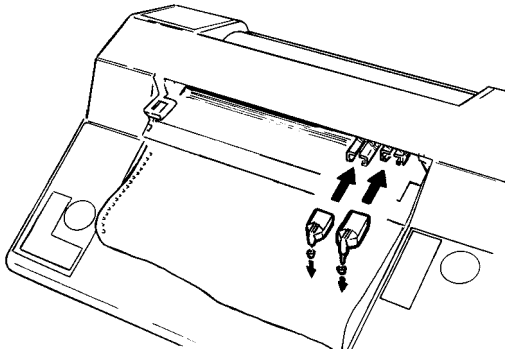
1 Remove the cap.

2 Gently push the pen into the plastic pen holder (2.), avoiding sidewards or upwards pressure.

Note: *To prevent the pens from drying when the recorder is not in use, cover the tips of the pens using the caps provided.*

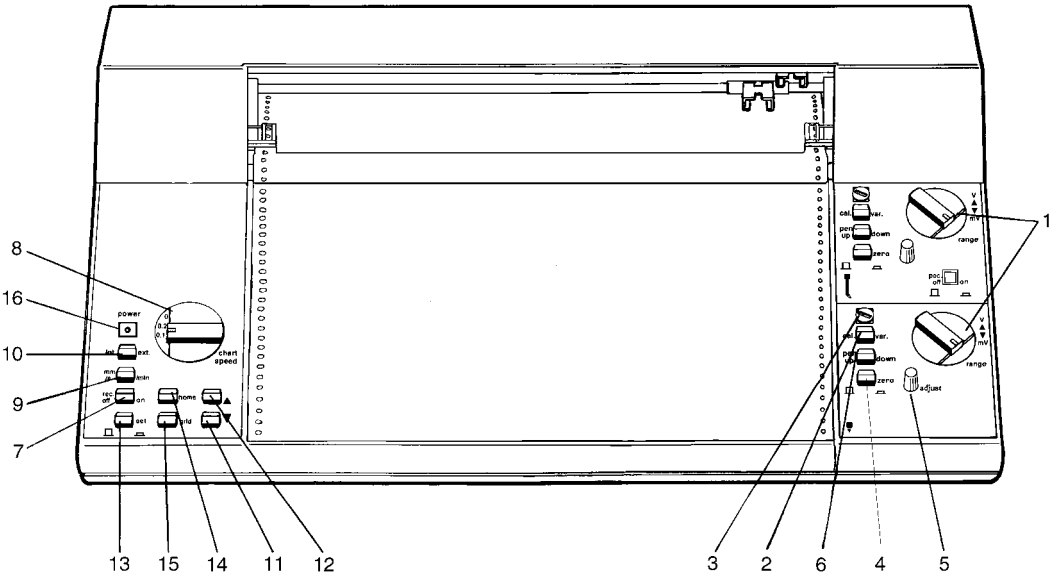


1.



2.

8.6.4 Preparing the recorder for a run



1 Turn on the mains power to the recorder.

2 Set the **zero** keys (4) to the **down** position.

3 Select a suitable low chart speed (0.5–2 mm/min) with the **chart speed** selector (8) and the **mm/s–mm/min** key (9).

4 Set both **pens** (6) to position **down**. Use the **adjust** knob (5) to make a coarse zero adjustment to the right-hand zero on the chart.

5 Set **rec.** (7) to position **on** and make a final zero adjustment.

6 Set **rec.** to position **off.** Use the forward **feed** key (11, 12) to align the short-nib pen with a grid line and press the **set** key (13), i.e. set start position.

7 Set the **zero** keys to the **up** position.

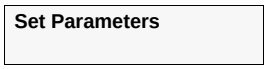
8 Select a suitable chart speed (2–20 mm/min) according to the length of the purification.

9 Select 1 V with the **range V/mV** selector (1).

10 The recorder is made ready to use by setting the **rec.** key to **on**. The chart paper starts rolling when the run starts.

8.6.5 Setting analog outputs

The **Set Analogue Out** menu is used to select which measurement parameter that should be associated to each channel. The menu is also used for setting measurement parameters (zero and full range values) for **UV**, **Cond**, **pH** and **Press** on the analogue output channels.

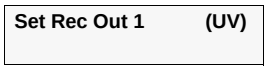


1 Select the **Set Parameters** menu in the main menu and press **OK**.

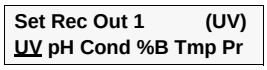
2 Select the **Setup Analogue Out** menu and press **OK** to enter the settings menu.

Setting parameters for the channels

UV, pH, conductivity, concentration of buffer B, temperature and pressure are measurement parameters that can be associated to the analogue output channels.



1 When entering the **Setup Analogue Out** menu, the setting for channel 1 is displayed first. Press the up and down buttons to display the settings for channels 2 and 3.

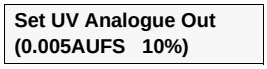


2 Select the desired channel and press **OK**. In this example, channel 1 is selected.

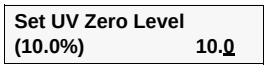
3 Select the desired parameter and press **OK**.

Note: *The analogue output level for **Tmp** has a fixed set value; 0 °C corresponds to 0 V and 50 °C corresponds to 1.0 V.*

Setting the UV analogue output

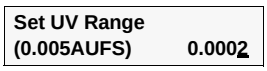


1 Select the **Set UV Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full range values). Allowed full range values are 0.0001, 0.0002, 0.0005, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0. Zero level is set as a percentage of full scale.



2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.

3 Set the desired zero level value. Press **OK** to acknowledge.



4 Press the down button to access the settings menu. The current setting is displayed. Press **OK**.

5 Set the desired full range value. Press **OK** to acknowledge.

Setting the Cond analogue output

Set Cond Analogue Out (00.00-50.00mS/cm)	1 Select the **Set Cond Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full scale values).
Set Cond Zero Level (0.00mS/cm) 0.00	2 Press **OK** to access the settings menu. The current setting is displayed. Press **OK**.
	3 Set the desired zero level value. The range is 0.000–999.9 mS/cm. Press **OK** to acknowledge.
Set Cond Full Scale (50.00mS/cm) 50.00	4 Press the down button to access the next settings menu. The current setting is displayed. Press **OK**.
	5 Set the desired full scale value. The range is 0.000–999.9 mS/cm. Press **OK** to acknowledge.
	6 Press **Esc** to return to the **Set UV Analogue Out** menu.

Setting the pH analogue output

Note: *The pH values for zero level and full scale must differ by at least 1 pH unit*.

Note: *The zero level and full scale values can be calibrated in any order.*

Set pH Analogue Out (pH 0.00-14.00)	1 Select the **Set pH Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. Current analogue settings are displayed (zero and full scale values).
Set pH Zero Level (pH 0.00) 00.00	2 Press **OK** to access the settings menu. Press **OK**.
	3 Set the desired zero level value. The range is pH -0.50–14.30. Press **OK** to acknowledge.
Set pH Full Scale (pH 14.00) 14.00	4 Press the down button to access the next settings menu. Press **OK**.
	5 Set the desired full scale value. The range is pH -0.50–14.30. Press **OK** to acknowledge.
	6 Press **Esc** to return to the **Set UV Analogue Out** menu.

Setting the Press analogue output

Set Press. Analogue Out (1.00 MPa) 1.00	1 Select the **Set Press Analogue Out** menu in the **Setup Analogue Out** menu by using the up and down buttons. The current analogue setting is displayed (full scale value).
Set Press. Analogue Out (2.00 MPa) 2.00	2 Press **OK** to access the settings menu. Set the desired full scale value. The range is 0.00–1.00 MPa. Press **OK** to acknowledge.

8.6.6 Printing curves directly after a run

Selected run data is usually printed directly during the run. However, if a re-print of the result is desired, this can be done as a post-run print-out. This procedure is described below.

The system has three analog output channels for printing the run data curves after the run. You can choose to print out UV absorbance, pH, conductivity, theoretical gradient (%B), temperature or pressure.

Memory Print Out?
(yes) yes no

When the run is completed, or if it has been aborted, the prompt **Memory Print Out?** is displayed.

1 To print out the run data, select **yes**.
Otherwise, select **no**.

Set Rec Out 1 (UV)
UV pH Cond %B Tmp Pr

2 At the **Set Rec Out 1** menu, select the parameter to be printed on channel 1.
Press **OK.**

Set Rec Out 2 (UV)
UV pH Cond %B Tmp Pr

3 At the **Set Rec Out 2** menu, select the parameter to be printed on channel 2.
Press **OK.**

Set Rec Out 3 (UV)
UV pH Cond %B Tmp Pr

4 At the **Set Rec Out 3** menu, select the parameter to be printed on channel 3.
Press **OK.**

Autoscaling
(no) yes no

5 If auto-scaling of the UV-curve is required, select **yes**.
Otherwise, select **no**.

6 Press the **set** key on the recorder to define the start position for the print-out.

7 Press **OK** to print the curves.

8.6.7 Printing curves before the next run

The system stores the run data from the latest run. Therefore, post-run print-out can be done either at the end of the run as described in the precious section, or before the next run using the **Set Parameters** menu as described below.

1 Set the **rec.** key on the recorder to **off**.

2 Press the **home** key to get the paper back to the start position.

3 Increase the **chart speed** value about ten times. This is due to that the curves are printed ten times faster at the post-run print-out than during the actual run.

4 Set the **rec.** key to **on** to enable the print-out.

5 Set the **pen** key to position **down**.

Set Parameters

6 Select main menu **Set Parameters** and press **OK**.

Memory Print Out

7 Select menu **Memory Print Out** and press **OK**.

Print out to
Recorder **Computer**

8 At the **Print out to** menu, select **Recorder** and press **OK**.

9 Select the desired parameters to be printed as in steps 2–5 on page 117.

The print-out will now overlay the previous curves.

10 Repeat the procedure above to select other parameters and print out the curves.

More information about the recorder is found in the *Recorder REC 112 User Manual* supplied.

9 Maintenance

This chapter contains a periodic maintenance schedule and instructions for maintenance, replacing components, and calibrations.

9.1 Periodic maintenance

Regular maintenance will help to keep your ÄKTAprime plus running smoothly. Follow the recommendations in this chapter to keep the system in good working order.

WARNING! Remove liquid or dirt from the system surface using a cloth and, if necessary, a mild cleaning agent.

WARNING! Always disconnect the power supply before attempting to replace any item on the system during maintenance.

WARNING! If there is a risk that spilt liquid have penetrated the casing of the instrument and come into contact with the electrical components, immediately switch off the system and contact an authorised service technician.

WARNING! When using hazardous chemicals, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the chemicals used. Follow local regulations and instructions for safe operation and maintenance of the system.

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

WARNING! NaOH is injurious to health. Avoid spillage.

WARNING! Only spare parts that are approved or supplied by GE Healthcare may be used for maintaining or servicing the system.

WARNING! Use ONLY tubings supplied by GE Healthcare to ensure that the pressure specifications of the tubings are fulfilled.

WARNING! If the system is turned, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections causing leakage.

9.1.1 Preventive maintenance schedule

Interval	Action
Daily	
System	• Inspect the complete system for eluent leakage. • The system can be left filled with buffer overnight. If you are not using the separation unit for a few days: 1. Wash the flow path with distilled water. 2. Remove the column and the pH electrode (optional). 3. Wash the flow path with 20% ethanol and store it in 20% ethanol. Make sure that all tubing and all flow paths used are rinsed.
Pump	• Check for leakage. If there are signs of liquid leaking from the pump: – Check the tubing connections. – Check the O-rings in the connection part. Replace the O-rings if necessary. • If there are signs of erratic or pressure pulsation, flush the pump with 100% methanol and then with distilled water.
pH electrode	• Calibrate the pH electrode according to the section Calibrating the pH electrode (optional).
Every week	
Inlet filters	• Check the inlet filters visually and replace them if necessary.
Every month	
Monitor	• Check the monitor according to section 9.5.
Flow restrictor	• Check that the flow restrictor generates the following back-pressure: 0.2 ± 0.05 MPa. Check the back-pressure as follows: 1. Disconnect the flow restrictor 2. Connect a capillary to port 1 of the injection valve. 3. Run the pump manually at 10 ml/min with water. Note the backpressure on the display. 4. Connect the flow restrictor to the open end of the capillary. 5. Run the pump at 10 ml/min with water. Note the back-pressure on the display. 6. Calculate the backpressure generated by the flow restrictor. Replace it if it is not within limit.

Interval	Action
Every 6 months or more often if required	
Monitor	• Clean the UV flow cell according to section 9.9 or 9.10. • Change the pH electrode. Refer to section 8.5.
Fraction collector	• Check the drive sleeve on the tube rack. Replace if worn. • Check the number of tube shifts according to section 9.7.
Superloop	• Check that the top, bottom and moveable seal O-rings are in good condition. Replace if necessary. • Check that the bottom end pieces are clean and undamaged.
Mixer	• Check that the mixer chamber is clean and without damage. • Check the tubing connectors. Replace if required. Refer to section 9.17.
Yearly	
Injection valve and buffer valve	• Check for external or internal leakage. Replace channel plate and distribution plate when required. Refer to section 9.13.
Every 2 years	
Mixer	• Replace the complete mixing chamber. Refer to section 9.17.
Superloop	• Replace O-rings. Refer to section 4 in the instruction supplied with Superloop.
When required	
Monitor	• Clean the conductivity flow cell according to section 9.11. • Clean the pH electrode flow cell according to section 9.18.
Pump	• Replace the check valve O-rings according to section 9.16. • Clean the check valves. If necessary, replace the check valve O-rings or the entire check valves according to section 9.16.

9.2 Cleaning the system

- Wipe the surface regularly with a damp cloth. Do not allow spilt liquid to dry on the instrument.
- Remove dirt from the surface using a cloth and a mild cleaning agent.
- Let the system dry completely before using it.

9.3 Cleaning the system flow path

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

Section 9.3.3 Monthly cleaning describes how to flush the system with a bacteriostatic solution.

Usually the column should be by-passed with a piece of i.d. 0.75 mm PEEK capillary before cleaning the system flow path. If not, make sure that the column withstands the expected flow and pressures.

For column cleaning procedures and storage instructions, please refer to the column instructions.

9.3.1 Betweens runs

Buffers not containing any salt can be left in the system for a short time after a run, even overnight (not in the pH electrode, see instructions below).

If a buffer containing salt has been used, the flow path should be flushed with deionized water. This is especially important if an organic solvent will be used in the next run.

To flush the flow path:

1 Fill a syringe with five times the sample loop volume of deionized water.

2 Rinse the sample loop by injecting the water through the fill port on the injection valve.

3 Put all used inlet tubings in water

4 In the **Templates** menu, select **Application Template** and then **System Wash Method**.

5 Select the used inlet ports. Inlets **A1** and **B** will always be washed.

6 Press **OK** to start the method. The system flow path is automatically flushed.

9.3.2 Weekend and long-term storage

CAUTION! Never leave the pH electrode in the electrode holder for any period of time when the system is not used, since this may cause the glass membrane of the electrode to dry out. Store the pH electrode fitted in the end cover filled with a 1:1 mixture of pH 4 buffer and 2 M KNO_3. Do NOT store in water only!

If you are not using the system for a few days or longer:

1 Wash all tubing and flow paths used with deionized water, for example by running the **System Wash Method** with all tubing inlets in water.

2 Replace the column with a bypass capillary.

3 Replace the pH electrode (optional) with a dummy pH electrode.

4 Wash the system with 20% ethanol and store it in 20% ethanol.

The UV flow cell can also be stored dry by flushing as above with distilled water and then 20% ethanol through the flow cell. Replace the red protective caps. Never use compressed air as this may contain droplets of oil.

pH electrode (optional)
The pH electrode should always be stored in a 1:1 mixture of pH 4 buffer and 2 M KNO_3 when not in use. After removing the pH electrode from the flow cell, insert a dummy electrode in the flow cell.

9.3.3 Monthly cleaning

WARNING! NaOH is injurious to health. Avoid spillage.

Clean the system every month, before service and maintenance, or when problems, such as ghost peaks occur. The system is cleaned as follows:

1 Disconnect the column and replace it with a suitable capillary.

2 Put all tubing inlets in 1 M NaOH.

3 Run **System Wash** method for all inlet tubings.

4 Flush the whole system with 1 M NaOH for 20 minutes (1 ml/min).

5 Immediately repeat steps 3 and 4 with distilled water to rinse the system of NaOH.

9.3.4 Other cleaning considerations

After repeated separation cycles, contaminating material may progressively build up in the system and on the columns. This material may not be removed by the cleaning step described above. The nature and degree of contamination depends on the sample and the chromatographic conditions employed.

9.4 Moving the system

CAUTION! Never lift the system by the components mounted on the system chassis.

WARNING! When using hazardous chemicals, make sure that the entire system has been flushed thoroughly with bacteriostatic solution, e.g. NaOH, and distilled water before service and maintenance.

WARNING! If the system is turned, the external capillaries and other tubing may become entangled in nearby objects and be pulled from their connections causing leakage.

Before moving the system, ensure that all cables and capillaries connected to peripheral equipment and liquid containers are disconnected.

Lift the system by placing your fingers in the gap between the base and the work bench surface, grasping firmly and lifting.

9.5 Checking the UV monitor

9.5.1 Checking lamp intensity

Check Lamp Intensity
R 215.5 S 214.7mV

1 Select menu **Check** and press **OK**.

2 Select menu **Check Lamp Intensity**.

If:
R<300mV for 254 nm,
R<150mV for 280 nm, or
R<150mV for 214 nm,
replace the lamp assembly according to section 8.2.5 Changing the lamp assembly (optional), or contact GE Healthcare for lamp replacement.

9.5.2 Checking lamp run time

Check Lamp Run Time
Hg 2300h Zn 340h

1 Select menu **Check** and press **OK**.

2 Select menu **Check Lamp Run Time**.

- The lifetime of a Hg lamp at 254 nm is typically 7000 hours in room temperature (in coldroom, typically 2000 h).
- The lifetime of a Hg lamp at 280 nm is typically 3500 hours in room temperature.
- The lifetime of a Zn lamp is typically 2000 hours in room temperature.

When necessary, replace the lamp assembly according to section 8.2.5 Changing the lamp assembly (optional), or contact GE Healthcare for lamp replacement.

9.5.3 Checking autozero

The internal absorbance value for autozero can be checked to test the consistency of buffers.

1 Select menu **Check** and press **OK**.

Check Autozero
AZ 0.0001 AU

2 Select menu **Check Autozero**. The autozero absorbance value for the wavelength used is shown.

9.6 Checking the pump

9.6.1 Checking pump run time

1 Select menu **Check** and press **OK**.

Check Pump Run Time
00014h

2 Select menu **Check Pump Run Time**.

The lifetime of the wearing parts (glass tube, piston, sealings and O-rings) is typically 2000 hours at normal use.

When necessary, contact GE Healthcare for replacement.

9.6.2 Checking pumped volume

1 Select menu **Check** and press **OK**.

Check Pumped volume
194529452ml

2 Select menu **Check Pumped Volume**.

When required, contact GE Healthcare for replacement.

9.7 Checking the fraction collector

9.7.1 Checking tube shifts

Check Tube Shifts
17564

1 Select menu **Check** and press **OK**.

2 Select menu **Check Tube Shifts**.

When required, contact GE Healthcare for replacement.

9.8 Checking the rotary valves

Check Valve Shifts
BV:17564 IV:28143

1 Select menu **Check** and press **OK**.

2 Select menu **Check Valve Shifts**.

The value after **BV** shows the number of buffer valve shifts. The value after **IV** the number of injection valve shifts. One shift means the shifting between two adjacent positions. The lifetime of the valves is > 50 000 shifts.

When necessary, replace the plates according to section 9.13 Replacing plates in the rotary valves, or contact GE Healthcare for sealing replacement.

9.9 Cleaning the UV flow cell in-place

WARNING! NaOH is injurious to health. Avoid spillage.

Pump a cleaning or sanitizing agent through the flow cell. The standard recommendation is to pump 1 M NaOH for 30 minutes and then wash out immediately with buffer.

9.10 Cleaning the UV flow cell off-line

A clean flow cell is essential for correct operation of the UV monitor.

CAUTION! Do not allow solutions that contain dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell as damage to the flow cell may occur.

1 Connect a syringe to the inlet of the flow cell and squirt distilled water through the cell in small amounts. Then fill the syringe with a 10% surface active detergent solution like Decon 90, Deconex 11, RBS 25 or equivalent, and continue to squirt five more times.

2 Leave the detergent solution in the flow cell for at least 20 minutes.

3 Pump the remaining detergent solution through the flow cell.

4 Rinse syringe and flush the flow cell with distilled water (10 ml).

9.11 Cleaning the conductivity flow cell off-line

WARNING! NaOH is injurious to health. Avoid spillage.

If the conductivity measurements are not comparable to previous results, the electrodes in the flow cell may be contaminated and require cleaning. To clean the flow cell:

1 Pump 15 ml of 1 M NaOH at 1 ml/min through the flow cell either by using the system pump or a syringe.

2 Leave for 15 minutes.

3 Rinse thoroughly with degassed distilled water.

Note: *If the flow cell is totally blocked, the blockage can be removed using a needle or a wire with a diameter less than 0.8 mm.*

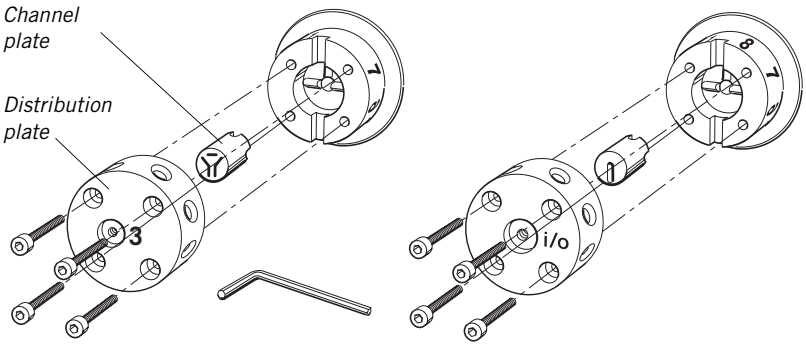
9.12 Replacing the conductivity cell

The conductivity flow cell can be replaced when required. Make sure the system is switched off before connecting/disconnecting the cell from the rear of the system.

If the cell is replaced with a new flow cell, the system must be calibrated with the new cell constant written on the flow cell package. See section 9.20.3 Calibrating conductivity. If the cell constant is not known, it can be determined.

9.13 Replacing plates in the rotary valves

A replacement kit for each valve is available. Refer to 11.5 Ordering information.



Injection valve *Buffer valve*

1 Make sure that the valve is in position 1 (see figure) and then disconnect all tubings.

2 Remove the four screws on the front using a 3 mm hex wrench. Loosen each one equally in turn so that the distribution plate comes off parallel to the valve body.

3 Slide the screws out.

4 Remove the distribution plate containing the ports.

5 Remove the old channel plate and insert the new one.

6 Remount a new distribution plate so that the text **3** (injection valve) or **i/o** (buffer valve) is horizontal and to the right of the central tubing connection. Using a hex wrench, tighten the four screws in turn, a little at a time, until the distribution plate is fixed to the valve body.

9.14 Removing and assembling the pump

The connection part should be removed to allow access to the check valves and the O-rings.

9.14.1 Removing the connection part

Before disassembling the pump, move the input liquid bottle below the level of the pump to prevent siphoning.

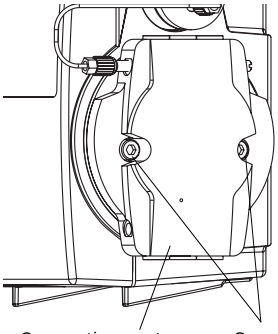
1 Turn off the system with the mains power switch.

2 Disconnect the inlet and the outlet tubing from the connection part.

3 Release the pump from the system.

4 Unscrew the two attachment screws using a 4 mm hex wrench.

5 Remove the connection part.



9.14.2 Installing the connection part

1 Wipe the back of the connection part and the other parts behind the connection part with a clean cloth.

2 Check that none of the seven O-rings on the rear side of the connection part has come loose.

3 Fit the connection part in position. Fasten the two attachment screws using the 4 mm hex wrench.

4 Reconnect the inlet and the outlet tubing.

5 Purge the pump carefully and check that the fault is corrected. See section 5.2.2 Purging pump and inlet tubing.

9.15 Replacing O-rings in the pump

9.15.1 Required spare parts

- O-ring kit (see Ordering information for code no.)

9.15.2 Replacing an O-ring

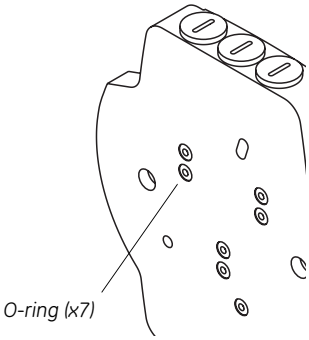
To replace an O-ring:

1 Remove the connection part according to section 9.14.1 Removing the connection part.

2 Remove the faulty O-ring carefully to avoid making scratches on the connection part.

3 Fit the new O-ring in position.

4 Reinstall the connection part according to section 9.14.2 Installing the connection part.



9.16 Cleaning and replacing check valves in the pump

Faulty operation of the check valves is usually indicated by irregular flow, very low flow, or unstable pressure traces. Probable causes are air, dirt, or a damage in a check valve preventing it from closing to seal and hold the pressure. Liquid appearing at a banjo fitting might indicate that a check valve O-ring is damaged.

9.16.1 Required spare parts

- Check valve (there are four different check valves. See Ordering information for code nos.)
- O-ring kit (see Ordering information for code no.)

9.16.2 Cleaning the check valves in-place

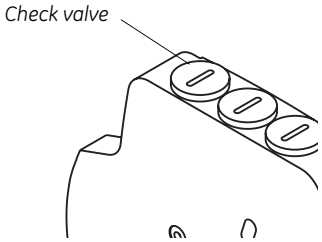
Try to clean the check valves in-place by pumping 100% ethanol for approximately 10 min. If this does not correct the problem, follow the instructions below to remove and then clean the valves. If necessary, a check valve or O-rings might need to be replaced.

9.16.3 Removing the check valves

If the condition of the check valve is not improved by in-place cleaning, remove it as follows:

1 Remove the connection part according to section 9.14.1 Removing the connection part.

2 The check valves in the connection part are locked in position with banjo fittings. Remove the check valves using the screwdriver.



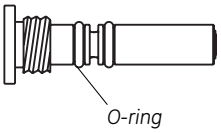
9.16.4 Cleaning the check valves off-line

1 Immerse the check valves in 100% ethanol and place in an ultrasonic bath for 5–10 minutes.

2 Repeat the ultrasonic bath with distilled water.

9.16.5 Replacing the check valve O-rings

Liquid appearing at a banjo fitting might indicate that a check valve O-ring is damaged. It might also cause reduced flow or pressure fluctuation.



Carefully replace both O-rings on the check valve with new ones if you suspect that an O-ring is damaged.

9.16.6 Installing the check valves

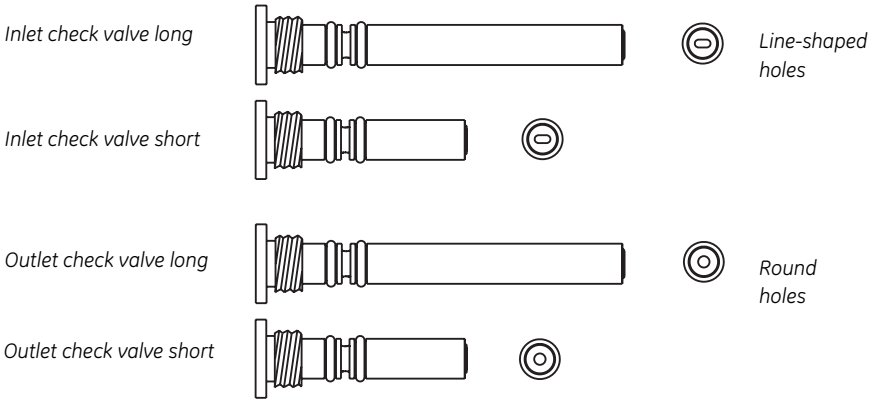
If cleaning of the check valve as described in section 9.16.4 Cleaning the check valves off-line does not correct the fault, replace the check valve with a new one.

Note: *Make sure that the check valves are installed in their correct positions. By mistake, an inlet check valve can be installed incorrectly in an outlet check valve position, and vice versa.*

The check valves are of four different types:

- Inlet check valve short (1 pc.)
- Inlet check valve long (2 pcs.)
- Outlet check valve short (2 pcs.)
- Outlet check valve long (1 pc.)

The inlet check valves have line-shaped holes.
The outlet check valves have round holes.



To install a check valve:

1 Carefully insert the check valve fully.

Note: *Make sure that the inlet check valves are installed next to the inlet (lower) port on the connection part, and the outlet check valves next to the outlet (upper) port.*

2 Fasten the check valve using the screwdriver.

3 Reinstall the connection part according to section 9.14.2 Installing the connection part.

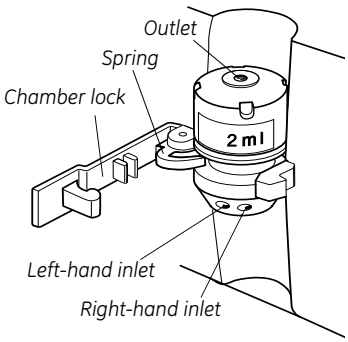
9.17 Replacing mixer chamber

WARNING! When using hazardous chemicals, make sure that the entire system flow path has been flushed thoroughly with distilled water before maintenance.

1 Make sure the pump is stopped.

2 Place the buffer bottles lower than the mixer to prevent draining, and then remove the inlet and outlet tubing.

3 Open the chamber lock holding the mixer chamber. A spring is securing the chamber in position when the lock is opened.



4 Pull out the mixer chamber gently.

5 Move the stop plug to the right-hand inlet of the new mixer chamber.

6 Insert the new mixer chamber and close the lock.

7 Replace the inlet and outlet tubing.

9.18 Cleaning the pH electrode (optional)

Note: *The pH electrode has a limited lifetime and should be replaced every six months, or when the response time is slow or the slope is out of range (<80%).*

WARNING! NaOH is injurious to health. Avoid spillage.

Use one of the following procedures to clean the electrode to improve the response:

- **Salt deposits**: Dissolve the deposit by immersing the electrode, first in 0.1 M HCl, then in 0.1 M NaOH, and again in 0.1 M HCl. Each immersion is for a period of 5 minutes. Rinse the electrode tip in distilled water.
- **Oil or grease films**: Wash the electrode tip in liquid detergent and water. If the film is known to be soluble in a particular organic solvent, wash with this solvent. Rinse the electrode tip in distilled water.
- **Protein deposits**: Dissolve the deposit by immersing the electrode in a 1% pepsin solution in 0.1 M HCl for five minutes, followed by thorough rinsing with distilled water.

It these procedures fail to rejuvenate the electrode, the problem is most likely a clogged liquid junction. Use the following procedure:

1 Heat a 1 M KNO_3 solution to 60-80 °C.

2 Place the electrode tip in the heated KNO_3 solution.

3 Allow the electrode to cool while immersed in the KNO_3 solution before re-testing.

If these steps fail to improve the electrode response, replace the electrode.

9.19 Replacing the pH electrode (optional)

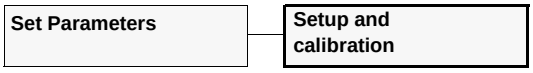
See section 8.5 pH flow cell and electrode (optional).

9.20 Calibrations

Follow the calibration schedule below to maintain accurate measurements.

Component	How often
Pump	Only necessary after replacing spare parts.
Pressure offset	When required.
Conductivity flow cell	
Cell constant	Only necessary if specific conductivity with high accuracy is measured.
Temperature	Must be done when changing the flow cell.
Entering a new cell constant	Must be done when changing the flow cell.
pH electrode (optional)	Every day

The calibrations are made in the **Setup and calibrations** menu.



9.20.1 Calibrating the pump

Calibrating the flow rate

Calibrate the pump after replacing spare parts.

1 Make sure no bubbles are trapped in the flow path.

2 Immerse the inlet tubing A1 in a vessel filled with degassed buffer.

3 Place the waste tubing from port 5 on the injection valve in an empty vessel (the flow will automatically be diverted to port 5 during the calibration).

4 Select **Start Pump Calibration** in **Setup and calibration** menu. Press **OK**.

5 Enter the intended flow rate as calibration flow rate and press **OK**.

6 Measure the volume of the water collected in the vessel.

7 Enter the measured volume in ml in the **Enter Collected Volume** menu and press **OK**.

8 At the **Pump Calibrated OK** menu press **OK**.

9.20.2 Calibrating the pressure sensor

Calibrating the pressure offset
The zero pressure should be calibrated when required.

1 Make sure that the pressure sensor is exposed to atmospheric pressure only, i.e. no backpressure.

Change Press Offset (1005 mV)

2 Select the **Change Press Offset** menu in the **Setup and calibration** menu. Press **OK**.

Set zero pressure to calib. Press OK

3 At the **Set zero pressure to calib**. menu press **OK**.
The calibration only takes a few seconds.

4 Press **OK** at the **Calibrating Offset Done!** menu.

9.20.3 Calibrating conductivity

Set up adjust cell constant
After replacing the flow cell, the cell constant has to be set. The cell constant is written on the cell packaging.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration** and press **OK**.

Setup Cond

3 Select sub menu **Setup Cond** and press **OK**.

Set Adj Cell Const ($83.56 cm^{-1}$)

4 Select sub menu **Set Adj Cell Const**. The current cell constant is shown. Press **OK**.

Warning! This will change cell calibr.

5 A warning message is shown until confirmed by pressing **OK**.

Set Adj Cell Const ($83.56 cm^{-1}$) 83.55

The current cell constant is displayed as default. Enter the new cell constant as read from the packaging and press **OK**. The range is 0.1–300.0 cm^{-1}.

Set up adjust conductivity

Normally, it is not necessary to adjust the cell constant because the flow cell is pre-calibrated on delivery. Adjustment is only necessary when replacing the conductivity flow cell with a flow cell whose cell constant is unknown. We recommend that the conductivity flow cell is recalibrated after cleaning.

Note: *The conductivity temperature compensation must not be used when adjusting the cell constant. Set the* ***Set Cond Temp Comp*** *to 0 (see page 138). The temperature sensor must be calibrated before adjusting the cell constant (see page 139).*

1 Prepare a calibration solution of 1.00 M NaCl, 58.44 g/l. Let the solution stand until it is at room temperature. This is important for exact measurements.

2 Fill the flow cell completely with the calibration solution by pumping at least 15 ml through the cell with a syringe.

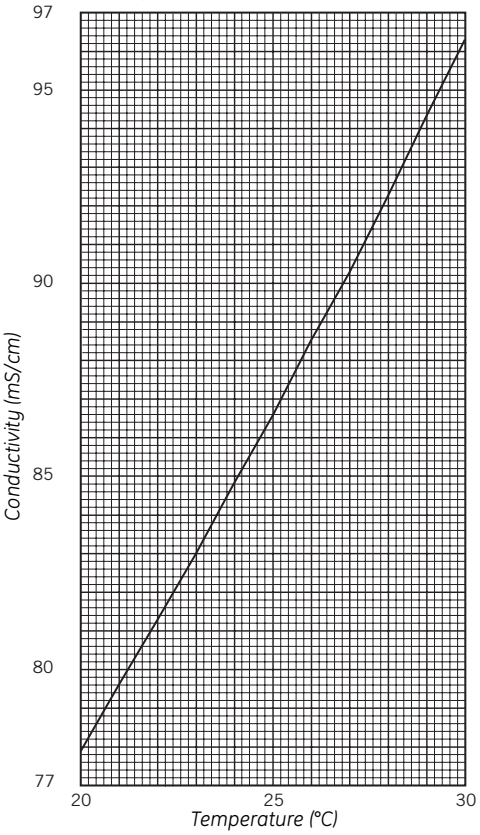
3 Stop the flow and wait 15 minutes until the temperature is constant in the range 20–30 °C.

4 Read the conductivity value displayed and compare it with the theoretical value from the graph below at the temperature of the calibration solution. If the displayed value and the theoretical value correspond, no further action is required.

If the values differ, proceed with the actions below.

Set Parameters	5 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	6 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	7 Select sub menu **Setup Cond** and press **OK**.
Set Adjust Cond (80.32mS/cm)	8 Select sub menu **Set Adjust Cond**. The current conductivity value is shown. Press **OK**.
Warning! This will change cell calibr.	9 A warning message is shown until confirmed by pressing **OK**.
Set Adjust Cond (80.32mS/cm) 83.55	10 The current value is displayed as default. Enter the theoretical conductivity value according to the graph and press **OK**. The new cell constant is automatically calculated. The range is 1.000–999.9 mS/cm.

Conductivity of 1.00 M NaCl at 20–30°C



Set up conductivity temperature compensation

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Cond Temp Comp (0.0%)	4 Select sub menu **Set Cond Temp Comp** and press **OK**. The current temperature compensation factor is shown. 0.0% means that the compensation is off (default setting). The range is 0.0–9.9%.
Set Cond Temp Comp (0.0%) 0.0	5 The current compensation factor is displayed as default. Adjust the compensation factor setting as necessary and press **OK**.

Set up conductivity reference temperature

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Cond	3 Select sub menu **Setup Cond** and press **OK**.
Set Cond Ref Temp (25.0°C)	4 Select sub menu **Set Cond Ref Temp** and press **OK**. The current reference temperature value is shown. 25 °C is the default setting. The range is 0.00–99.9 °C.
Set Cond Ref Temp (25.0°C) 25.0	5 The current reference temperature value is displayed as default. Adjust the reference temperature value setting as necessary and press **OK**.

9.20.4 Calibrating the temperature sensor

Calibration of the temperature sensor in the conductivity flow cell is only necessary if the monitor is used in high accuracy measurement or if the conductivity flow cell is replaced.

1 Place the flow cell together with a precision thermometer inside a box or empty beaker to ensure that they are not exposed to draught. Leave them for 15 minutes to let the temperature stabilize.

2 Read the temperature on the thermometer.

Set Parameters	3 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	4 Select sub menu **Setup and calibration** and press **OK**.
Setup Temp	5 Select sub menu **Setup Temp** and press **OK**.
Set Adjust Temp (25.0°C)	6 Select sub menu **Set Adjust Temp**. The current temperature is shown. Press **OK**.
Warning! Temp calibr will be changed.	7 A warning message is shown until confirmed by pressing **OK**.
Set Adjust Temp (25.0°C) 25.0	The current adjustment value is displayed as default. Enter the temperature shown on the thermometer and press **OK**.

9.20.5 Calibrating the pH electrode (optional)

A good laboratory routine is to calibrate the pH measurement once a day, when the electrode is replaced or if the ambient temperature changes. The pH electrode is calibrated using standard buffer solutions in a two point calibration. The two buffer solutions can have any pH value as long as the difference between them is at least 1 pH unit. The calibration procedure can be done with the pH electrode either fitted in or removed from the flow cell.

Calibrating with the electrode outside the flow cell

When calibrating the electrode out of the flow cell and changing from one buffer to another, rinse the electrode tip with distilled water and dab it carefully with a soft tissue to absorb the remaining water. Do NOT wipe the electrode as this may charge it and give unstable readings.

The steps below describe the procedure used with the electrode removed from the flow cell.

1 Remove the pH electrode from the flow cell and immerse the electrode in the first standard buffer solution (normally pH 7.0).

Set Parameters

2 From the main menu, select menu **Set Parameters** by pressing the up or the down button. Press **OK**.

Setup and calibration

3 Select menu **Setup and calibration**. Press **OK**.

Setup pH

4 Select menu **Setup pH** and press **OK**.

Calibrate pH (7.00 - 12.00)

5 Select menu **Calibrate pH**. Current calibration values are displayed (buffer 1 – buffer 2).
Buffer 1 = fixed lower calibrated pH value. Range=0.00-14.00
Buffer 2 = fixed higher calibrated pH value. Range=0.00-14.00

Note: *The values for buffer 1 and 2 must differ by at least 1 pH unit*.

Calibrate pH Buffer 1

6 Press **OK** to access the settings menu. The order of calibration, buffer 1 or buffer 2, is optional. Press **OK** to start with buffer 1, or press the down button to start with buffer 2. In this example, we start with buffer 1.

Calibrate pH Buffer 1 (7.00) Please wait!

7 This text disappears when the reading is stable and the following text is then shown:

Calibrate pH Buffer 1 (7.00) 7.00

8 Adjust the pH value in the display with the up and down buttons so that it corresponds to the known pH value of the first buffer solution. Press **OK**.

Calibrate pH Buffer 2

9 At the buffer 2 calibrating menu, rinse the electrode tip with distilled water and then immerse the electrode in the second buffer solution (e.g. pH 4.0 or 9.0). Then press **OK**.

Calibrate pH Buffer 2
(9.00) Please wait!

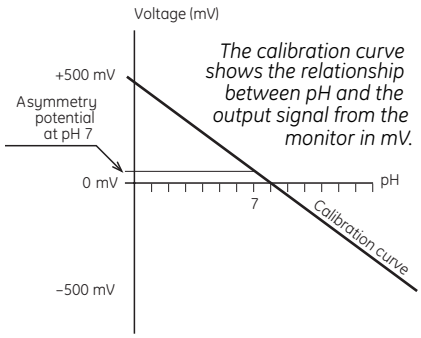
10 The text opposite disappears when the reading is stable and the text below is then shown.

Calibrate pH Buffer 2
(9.00) 9.00

11 Adjust the pH value in the display with the up and down buttons so that it corresponds to the known pH value of the second buffer solution. Press **OK**.

Calibrated Electrode
Slope 98.5% 9,5 mV

12 After the calibration with buffer 2, the system automatically enters the **Calibrated Electrode** menu. This menu shows the slope of the calibration curve, where 100% corresponds to 59.16 mV per pH step at 25°C. The asymmetry potential at pH 7 is shown as a mV value. Press **Esc** repeatedly to return to the **Set Parameters** menu.



13 Before use, rinse the electrode using distilled water.

A new electrode typically has a slope of 95–102% and an asymmetry potential within ±30 mV. As the electrode ages, the slope decreases and the asymmetry potential increases.

As a rule, when an electrode has an asymmetry potential outside ±60 mV and a slope lower than 80%, and no improvement can be made by cleaning, the electrode should be changed.

An electrode is still usable at lower slopes and higher asymmetry potentials but the response will be slower and the accuracy diminished.

Calibrating with the electrode in the flow cell

When calibrating with the electrode fitted in the flow cell, follow the above procedure but let at least 30–35 ml (with 2 ml mixer) of standard buffer solution be pumped through the system to stabilize pH. Leave the pump running while calibrating. Switch to the second standard buffer solution and repeat the procedure.

Set up pH temperature compensation

The relationship between pH and the output signal from the pH electrode is temperature dependent. For accurate measurements during temperature changes, the pH measurement can be temperature compensated. In normal applications, when the temperatures of the buffers and calibration buffers are identical, temperature compensation is not necessary.

When using temperature compensation, it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.

Set Parameters	1 From the main menu, select sub menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration**. Press **OK**.
Setup pH	3 Select sub menu **Setup pH** and press **OK**.
Set pH Temp Comp (off)	4 Select sub menu **Set pH Temp Comp**. The current setting for showing pH is displayed. If **on** is shown, **Tc** is displayed in the running display. If **off** is shown (default), **Tc** is not displayed. Press **OK** to change the setting.
Set pH Temp Comp (off) on <u>off</u>	5 Select the desired setting and press **OK**.

10 Troubleshooting

10.1 Faults and actions

This section lists faults observed with specific monitor measurements and the specific components. The faults are listed as follows:

Type	Page
System	144
UV curve	145
Conductivity curve	146
pH curve (optional)	147
Pressure curve.	144
Pump	148
Mixer	148
Fraction collector	149
Buffer valve and injection valve	149

If the suggested actions do not correct the fault, call GE Healthcare.

ÄKTAprime plus V 3.00

When contacting GE Healthcare for support, state the program version of the system, which is shown for a few seconds during start-up.

WARNING! The system should not be opened by the user. It contains high voltage circuits that can deliver a lethal electric shock.

10.2 System

Fault	Action
No text on the front display	1 Check that the mains cable is connected and that the power is turned on.

10.3 Pressure curve

Fault	Action
Pressure limit exceeded, inaccurate reading	1 Calibrate the pressure monitor. 2 Check that inlet or outlet tubings are not clogged. Replace if necessary. 3 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If the leakage persists, replace the assembly.
Irregular flow, noisy baseline signal, irregular pressure trace	Air bubbles passing through or trapped in the pump. 1 Check that there is sufficient eluent in the reservoirs. 2 Check all connections for leakage. 3 Use degassed buffers. 4 Remove any air bubbles according to section 10.13. Blockage or partial blockage of the flow path 1 Flush the flow path to clear the blockage. 2 If necessary, replace the tubing. 3 Check the inlet tubing filter. 4 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If the leakage persists, replace the assembly.

10.4 UV curve

Fault	Action
Noisy UV-signal, signal drift or instability	1 Select menu **Check Autozero** to check the autozero absorbance value. If the value is between 1.5 and 2, there may be air bubbles in the flow cell, or the wrong buffer system is in use. 2 Wrong filter for the lamp is being used. Check that the lamp is in the proper position and that the correct filter is used. 3 The buffer may be impure. Check if the signal is still noisy with water. 4 There may be air in the flow cell. Check that the flow restrictor generates a back-pressure of 0.2 ±0.05 MPa. Replace it if this is not within the limits. 5 If there is a lot of air in the water, degas the buffer before use. 6 Check the connections of the optical unit. 7 Clean the UV flow cell, see sections 9.9 and 9.10. 8 Locking nut in optical unit not properly tightened. Turn the locking nut to the stop position. 9 Air bubbles trapped in the pump. Refer to section 10.13.
Ghost peaks	1 Check that there is no air in the eluent. If necessary, degas the eluent 2 Clean the system in accordance with section 9.3. 3 Clean the column in accordance with the column instructions. 4 Check that the mixer is functioning properly and that the correct chamber volume is being used. 5 Unless you are using a low pressure column, try using a flow restrictor FR-904 instead of FR-902. This generates a higher back-pressure (0.4 MPa instead of 0.2 MPa).
Low sensitivity	1 Aging lamp. Check the lamp and replace if necessary. 2 Wrong lamp position. Check that the lamp position and the wavelength used (filter position) fit together.
Error in external chart recorder	1 Check the recorder according to the manufacturer's instructions.

10.5 Conductivity curve

Fault	Action
Incorrect or unstable reading	1 Check that the conductivity flow cell cable is connected properly to the rear of the system. 2 Check that the pump operates properly. 3 If temperature compensation is being used, check that the temperature sensor is calibrated, and that the correct compensation factor is used. 4 Check that the column is equilibrated. If necessary, clean the column. 5 Check the operation of the mixer.
Baseline drift or noisy signal	1 There may be air in the flow cell. Use a flow restrictor after the flow cell and check that the flow restrictor gives a back-pressure of 0.2 ±0.05 MPa. 2 Check for leaking tubing connections. 3 Check that the column is equilibrated. If necessary, clean the column. 4 Check the operation of the mixer and the pump. 5 Clean the flow cell according to the procedures in sections 9.11.
Conductivity measurement with the same buffer appears to change over time	1 Clean the flow cell according to the procedures in sections 9.11. 2 The ambient temperature may have changed. Use a temperature compensation factor.
Waves on the gradient	1 Check that the pump and the valves are operating properly and are programmed correctly. 2 Change to a larger mixing volume if necessary. 3 Check the operation of the mixer.
Absolute conductivity value is wrong	1 Turn the flow cell so the end with screws faces the pH flow cell. 2 Recalibrate the conductivity cell. 3 Calibration solution, 1.00 M NaCl, not correctly prepared. Prepare a new calibration solution and recalibrate the conductivity cell.
Ghost peaks appear in the gradient profile	1 A charged sample has been detected (e.g. protein). 2 Air bubbles are passing through the flow cell. Check for loose tubing connections. If necessary, use a flow restrictor after the conductivity cell.
Non-linear gradients or slow response to %B changes	1 Check that the tubing has been washed properly and that the pump is operating. 2 Change to a smaller mixer volume.

10.6 pH curve (optional)

Fault	Action
Incorrect/unstable pH reading	1 Check that the electrode cable is connected properly to rear of the system. 2 Check that the pump operates properly. 3 Check that the electrode is correctly inserted in the flow cell and, if necessary, hand-tighten the nut. 4 If air in the flow cell is suspected, tap the flow cell carefully or tilt it to remove the air. Alternatively, flush the flow cell with buffer at 20 ml/min for 30 s. Use a flow restrictor after the pH electrode. 5 Check that the pH electrode is not broken. 6 Check that the pH electrode is calibrated. 7 Check the slope. If it is outside the range 80–105% or if the asymmetry potential deviates more than ±60 mV from 0 mV, clean the pH electrode. Recalibrate. If the problem persists, replace the pH electrode. 8 Clean the pH electrode if required (see section 9.18). 9 Compare the response of the pH electrode with that of another pH electrode. If the response differs greatly, the electrode may require cleaning or replacement. 10 There may be interference from static fields. Connect the pH flow cell to the rear of the system using a standard laboratory 4 mm "banana plug" cable. 11 Check that the pH electrode has been calibrated at the correct temperature. 12 In organic solvents such as ethanol, methanol and acetonitrile, stable pH measurements are not possible since dehydration of the membrane will occur. We recommend that the pH electrode is not used in applications using organic solvents. 13 Clogged liquid junction. Refer to section 9.18.
No response to pH changes	1 Check that the electrode cable is connected properly to rear of the system. 2 The electrode membrane may be cracked. If so, replace the electrode.
Small response to pH changes	1 Clean the pH electrode according to section 9.18 and recalibrate. 2 If the problem persists, replace the pH electrode.

Fault	Action
Slow pH response or calibration impossible	1 Check the electrode glass membrane. If it is contaminated, clean the electrode according to the instructions in section 9.18. 2 If the membrane has dried out, the electrode may be restored by soaking it in buffer overnight. 3 Clogged liquid junction. Refer to section 9.18.
pH values vary with varied back-pressure	1 Replace the pH electrode.

10.7 Mixer

Fault	Action
Leakage	1 Check the tubing connections. Retighten or replace if necessary. 2 Check the mixer chamber. Replace if liquid has penetrated the mixer chamber walls and sealings.

10.8 Pump

Fault	Action
Irregular flow	1 Check the inlet and outlet tubings. 2 Remove any air bubbles according to section 5.2.2. 3 If still irregular flow, calibrate the pump according to section 9.20.1. 4 A check valve in the pump might be clogged or damaged. Remove the check valve according to section 9.14. Clean the check valve in an ultrasonic bath. If still irregular flow, replace the assembly.
Leakage	1 An O-ring in a check valve or in the connection part might be damaged. Examine the O-rings. If necessary, replace them according to the section 9.15.2 or 9.16.5.

10.9 Fraction collector

Fault	Action
No tube change	1 Press the **feed tube** key. If the motor does not start and an error appears, call GE Healthcare. 2 Push the delivery arm out to a safety stop. Press the **feed tube** key. If the motor starts, press the tube sensor together within 2 seconds. The motor should stop without an error code reported. If an errors appears, check the connection in the arm. If this is correct, the sensor or sensor connection are faulty. Call GE Healthcare.
Tubes skipped	1 The spring tension may be insufficient. Perform the actions described in section 10.12.
Drop synchronization is not functioning	1 Check that the drop synchronization function is turned on. See section 11.2.1. 2 The drop sensor photocell located above the tube sensor is dirty. Clean the photocell with a damp cloth. 3 Make sure that the fraction collection tubing is properly connected to the delivery arm. See section 8.4.5

10.10 Buffer valve and injection valve

Fault	Action
The valve is switching to wrong position	The valve parts may have been incorrectly reassembled after replacement. 1 Check that the distribution plate marking **i/o** (buffer valve) or **3** (injection valve) is horizontal.
External leakage	1 Check the tubing connections. Tighten or replace if required.
Internal leakage	Internal leakage can be detected at the small hole on the underside of the valve body. 1 Internal parts may be worn. Change channel plate and distribution plate according to section 9.13.
High back-pressure	1 Perform cleaning-in-place by flushing the system with detergent. 1 Change channel plate and distribution plate according to section 9.13.

10.11 Error messages

If the suggested actions do not correct the fault, call GE Healthcare.

Messages	Action
34 Start up failed Retry/Call service	1 Perform a new start-up. The preceding message may tell more about the cause 2 If not, call service.
35 WARNING wrong averaging time set	1 Wrong value for averaging time set. See section 11.2.1.
50 Electrical error Call for service ↓ **57 Electrical error Call for service** **75 Electrical error Call for service**	1 Call for service.
60 Tube switch always active	1 Check that the fraction collector is not stuck. 2 Check the tube indicator. 3 If the problem remains, call service.
61 No more tube is available	1 Put more tubes in the fraction collector.
62 Check that the tube position is OK	1 Check that a tube in the fraction collector touches the tube indicator. 2 Check the cable to the tube indicator. 3 If the problem remains, call service.
64 ERROR No drops check sensor	1 Check the cable to the tube indicator. 2 Check that it is dripping. If it flows continuously, reduce the flow or turn off the Drop sync function. 3 If the problem remains, call service.
65 ERROR Pump failure	1 Restart the system. 2 If the problem remains, call service.
66 Too short time between feeds	1 Reduce the flow or increase the fraction size.
67 ERROR Injection valve failure	1 Call service.
68 ERROR Buffer valve failure	1 Call service.

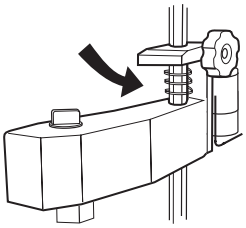
Messages	Action
69 ERROR Stop grad. set HOLD or PAUSE	1 Set the system in HOLD or PAUSE, or stop the gradient (%B).
70 Lamp disconnected If not, call service	1 Connect the lamp or call service.
71 WARNING low light intensity	1 Check the cables to the optical unit. 2 Check that lamp and filter position correspond. 3 Change lamp. If the problem remains, call service.
72 Change lamp or call service **76 Change lamp or call service**	1 If used in cold room, additional warm-up might be needed. 2 If the problem remains, change the lamp. 3 If the problem remains, call service.
73 WARNING Too much straylight leaks in	1 Check that the filter wheel cover is closed. 2 Check that non-transparent tubings are used at the UV flow cell inlet and outlet. 3 Check that the optical unit is not exposed to direct sunlight. 4 If the problem remains, call service.
77 WARNING Autozero out of range	1 Autozero is not allowed on a level above 2 AU. 2 Check buffers. 3 Clean UV flow cell.
78 ERROR Over pressure	1 Re-program the method.
79 ERROR Method corrupt in eeprom	1 Check which method is corrupted (erased). 2 If the problem remains, call service.
80 ERROR Reading from eeprom	1 Call service
81 ERROR Writing to eeprom	1 Call service.
82 ERROR Parameter fail in method	1 Re-program the method.
83 WARNING temp_cal will be changed **84 WARNING cond_cal will be changed**	1 Press OK to accept change. 2 Press ESC to skip the change.

Messages	Action
85 WARNING conscale (0-100%)<0.1mS	1 The difference between 0% and 100% must be at least 0.1 mS/cm. 2 Increase the span between zero and full scale setting. See section 11.2.1.
86 WARNING cond_cell bad/not connected	1 Check that the conductivity cell is connected. 2 Recalibrate temperature. 3 If the problem remains, replace the conductivity cell.
87 WARNING pH -probe bad/not connected	1 Check the pH electrode connection. 2 Clean the pH electrode. 3 If the problem remains, change the pH electrode.
88 Electrical error Call for service	1 Factory calibration for pH electrode is lost. The monitor can still be used but may not meet the specifications for pH measurements. 2 Call service.
89 Electrical error Call for service	1 Factory calibration for conductivity is lost. The monitor can still be used but may not meet the specifications for conductivity measurements. 2 Call service.
90 ATTENTION set<=0mV first **91 WARNING bad pH ad value**	1 Only visible to service personnel.
92 WARNING electrode slope <70 or >110% **93 pH _cal failed check electrode**	1 Electrode slope is out of range. Check buffers and recalibrate. 2 Clean the pH electrode and recalibrate 3 If the message remains, call service.
94 WARNING <1pH unit between cal_buff 1&2	1 The difference between the pH of the buffers used during calibration must be at least 1 pH unit.
95 Temp cal failed check cond cell	1 Check that the conductivity cell is connected. Recalibrate. 2 The measured temperature value differs from the reference value by more than ±5°C, or the actual temperature is lower than -8°C. Recalibrate.
97 WARNING pH scale (0-100%)<pH unit	1 The difference between the zero level and full scale must be at least 1 pH unit. Increase the span between zero and full scale settings. See section 11.2.1.
98 Cal failed. Cell constant not 0.1-300	1 Conductivity cell constant is out of range. 2 Wrong solution used during calibration. Use 1.00 M NaCl and recalibrate. 3 Air in conductivity cell during calibration. Flush the flow cell with calibration solution and recalibrate. 4 Dirty conductivity cell. Clean the flow cell and recalibrate. 5 If the problem remains, change the conductivity cell.

Messages	Action
99 ERROR Out of method memory	1 Maximum number of breakpoints in memory is 600. Delete a method to get more memory.
ERROR key	1 A key was pressed during self-test, or is faulty. 2 Switch off the system. 3 Switch on the system.
ERROR Number 102-104 **ERROR Number 109-113** **ERROR Number 119-121**	1 Switch off the system. 2 Check all connections. 3 Switch on the system.
Exc x/y in ab.c **Exc DIV/0 in ab.c** **Exc instr in ab.c** **Exc address in ab.c**	1 Switch off the system. 2 Check all connections. 3 Switch on the system.

10.12 Adjusting the spring tension of the delivery arm

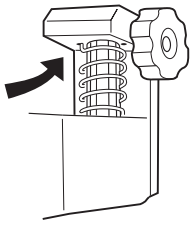
Incorrect spring tension can cause the fraction collector to skip tubes. The effect is greater as the arm moves towards the centre.



Spring tension is temperature sensitive. Low temperature, for example in a cold room, reduces the spring tension so it may be necessary to adjust the tension.

1 Remove the arm bracket from the stand.

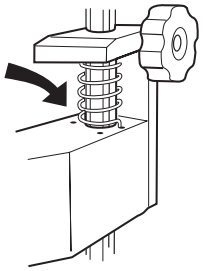
2 Dismantle the delivery arm from the bracket.



3 The top of the spring is fastened in one of two holes in the top of the arm bracket.

Looking at the arm bracket from the front of the unit, moving the spring from the right hand to left hand hole increases the tension, and conversely moving from the left hand to the right hand hole decreases the tension.

Hold the spring near the top and pull or prise it down and out of the top hole. Insert the spring in the other hole.



4 The bottom of the spring is fastened in one of four holes, equally spaced 1/4 turn apart. To adjust the bottom of the spring, hold it near the lower end and lift or prise the bottom of the spring out of the hole.

- To increase the tension turn the spring counter-clockwise.
- To decrease the tension turn the spring clockwise.

10.13 Removing trapped air bubbles

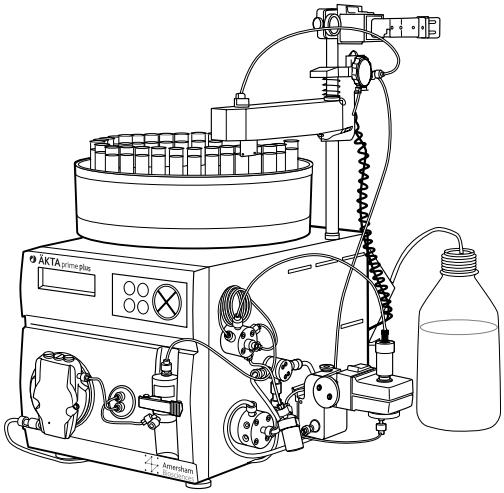
If there are large amounts of air in the tubing or if you suspect air in the pump, purge the flow path as described in section 5.2.2.

10.14 Restart after power failure

If the power to the system is interrupted, it automatically restarts when power is restored and displays the main operating menu. All set values and the data from the latest run are retained, and the lamp is switched on.

11 Reference information

11.1 System description



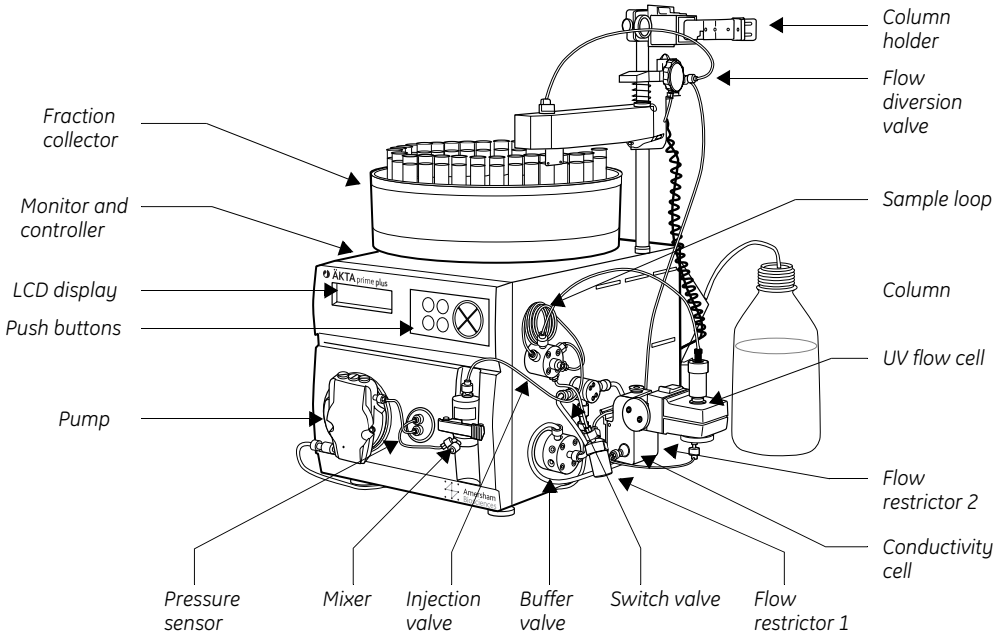
ÄKTAprime plus is a compact separation unit comprising components for fluid handling and for measuring UV-absorption, conductivity and pH (optional).

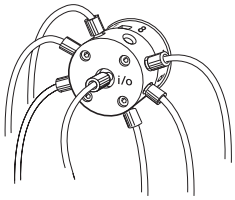
This section gives a brief description of the system and its components. It also describes optional components that may be connected to the system.

A built-in power control board supplies the components with power, and a controller handles the communication between the components via an internal high speed network.

11.1.1 Components description

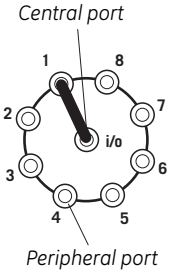
The following illustration shows the location of the components in the system.





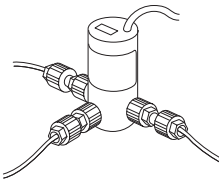
Buffer valve

This is a rotary valve which has 8 positions. The valve has a 360° rotating channel plate. As the plate is turned by the motor, the central port on the front is connected to one of the peripheral ports 1–8, allowing a clear liquid path. The valve switching is controlled by the system by reading the actual position of the channel plate.



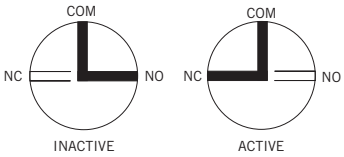
The buffer valve is used on the low pressure side in the flow path (before the pump). It it used for switching between sample and buffer solutions.

The switching parts are made of PEEK, which ensures long mechanical and chemical lifetime.

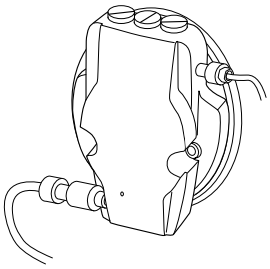


Switch valve/ Flow diversion valve

This is a 3-port/2-way valve, supplied with 24 V DC. It has one permanently open port marked COM and two ports marked NC (normally closed) and NO (normally open). Port COM may be used as an inlet or an outlet port.



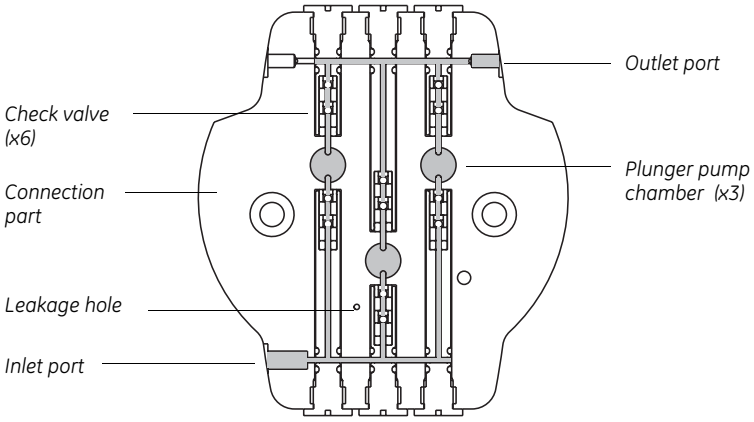
There are two switch valves in the system; one is used for gradient formation and the other one for flow diversion during fractionation.



Pump

The pump is equipped with a single input and double outputs (only one is used). It contains three internal plunger pumps which in combination with check valves create a smooth flow from the pump. The pump delivers flows up to 50 ml/min and pressures up to 1.0 MPa.

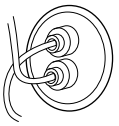
In the connection part, the inlet and outlet flow paths are split into three separate flow paths – one to/from each plunger pump chamber. All six flow paths are equipped with non-return check valves.



The plungers pump the liquid through the chambers while the check valves prevent the liquid from flowing backwards. The pump phases of three plunger pumps are displaced by 120° which results in a sequential motion of the plunger pumps, and thereby a smooth liquid delivery.

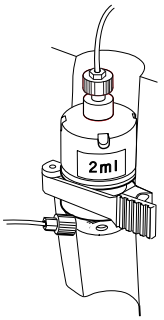
Leakage between the connection part and the pump chambers is prevented by O-rings. Any leakage behind the plungers is diverted through the drainage hole in the front of the connection part. The check valves are also equipped with O-rings.

The pump is equipped with automatic speed control to reduce pulsation and with pressure compensation.



Pressure sensor

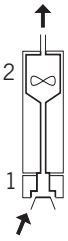
The pressure in the system is continuously measured by the pressure sensor located next to the pump. The pressure is shown on the display during the run. A maximum pressure limit can be set to protect the column.



Mixer

This is a dynamic, single chamber mixer with interchangeable mixer chambers. The system is delivered with a 2 ml chamber.

The eluents are mixed in two steps:



1 Premixing in a static mixer with a small volume (22 µl).

2 Dynamic mixing in a chamber with a rotating stirrer.

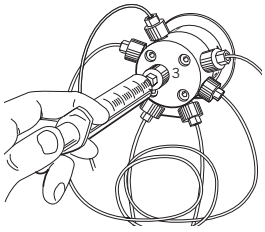
A mixer motor inside the system spins a magnet at 600 rpm, which causes the stirrer in the mixing chamber to rotate.

For optimal gradients at high flow rates, a larger mixer chamber is required. Other mixer chambers with 0.6, 5 and 12 ml mixer volumes are available as accessories.

When using eluents that are more difficult to mix, such as isopropanol and water, or if the pH and conductivity readings indicate uneven mixing of your buffers (unstable readings) a larger mixer volume will give better mixing.

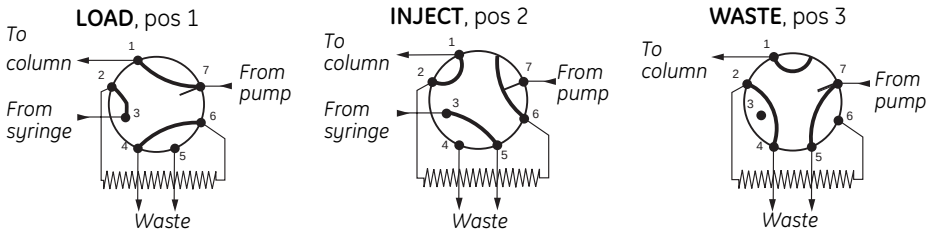
Injection valve

A 7-port motorized rotary valve is used as sample injection valve. It has a valve body with a rotating central core, the channel plate. As the channel plate is rotated by the motor, different ports are connected.



The valve has three different operating positions which make it possible to:

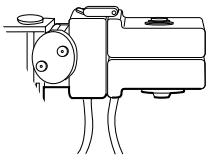
- Load a sample loop without disturbing column equilibration.
- Wash the sample loop while the column is in operation.
- Wash the pump for eluent exchange without disturbing the column.



The geometry of the valves ensures that the flow path is completely swept so that solvent or sample "memory effect" is virtually non-existing. The switching parts are made of PEEK, which ensures both long mechanical and chemical lifetime.

Monitor

This is a high precision on-line monitor for handling measurement data from the UV optical unit, the conductivity cell and the pH electrode (optional). In combination with the flow cells, the monitor offers fixed wavelengths of 214 nm (Zn-lamp, optional), 254 and 280 nm (Hg-lamp), fast response, high accuracy and reproducibility, and low dead volumes.



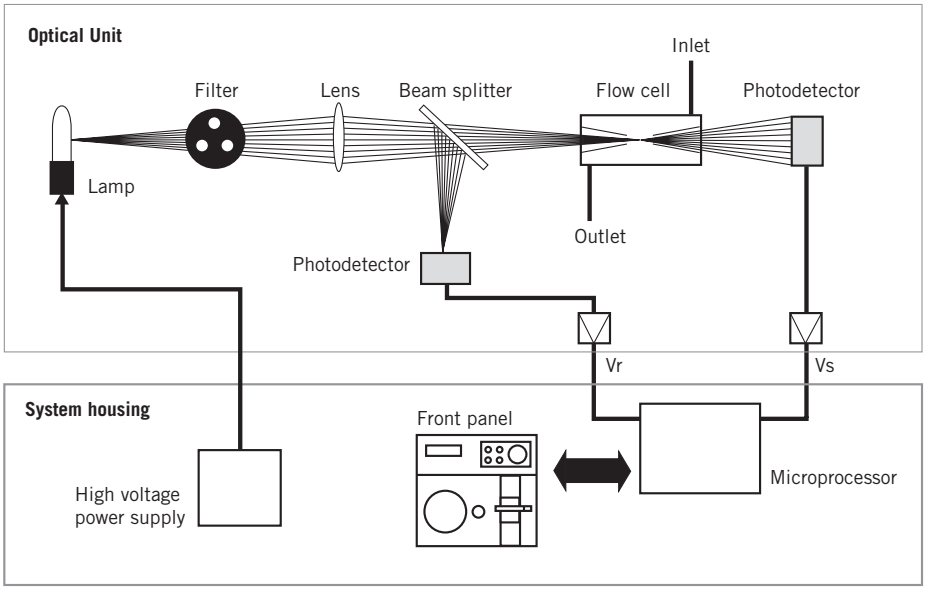
UV optical unit

The UV optical unit houses the lamp (Zn or Hg), the wavelength filter and the UV flow cell. There are two flow cells available; optical path length 2 mm or 5 mm (optional). The type of flow cell used depends on the sample amount applied and the size of the column.

UV flow cells with 2 and 5 mm path lengths

The light beam from the lamp is directed through a double conical or a straight flow-through cuvette (6 µl or 2 µl illuminated volume). The photodetector current is fed to the signal processing circuitry inside the system.

The reference signal comes from the same point in the lamp as the signal measuring the sample, thus assuring a stable baseline by eliminating the effects of variations in lamp intensity.

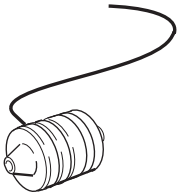


The Hg lamp emits light only at certain wavelengths. It does not emit light at 280 nm, so for this wavelength, the light is converted at a fluorescent surface before it passes the filter. On the lamp housing, there is a special exit for 280 nm light, which means that the lamp position needs to be changed when working with this wavelength.

For 214 nm wavelength, a Zn lamp is used. This lamp is larger than the Hg lamp and is therefore mounted in a larger lamp housing.

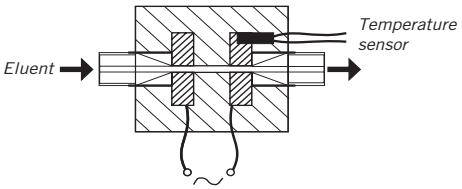
The lamp connectors are keyed to inform the monitor software which lamp type is connected.

Conductivity flow cell



The flow cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the eluent. The monitor controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz giving maximum linearity and true conductivity values.

The conductivity is automatically calculated by multiplying the measured conductance by the flow cell's cell constant. The cell constant is pre-calibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in section 9.20.3 Calibrating conductivity.

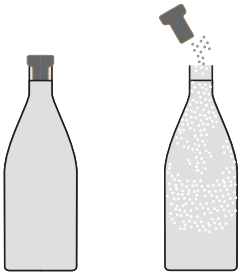


One of the electrodes has a small temperature sensor for measuring the temperature of the eluent in the flow cell. Temperature variations influence the conductivity and, in some applications when highly precise conductivity values are required, it is possible to program a temperature compensation factor that recalculates the conductivity to a set reference temperature.

Flow restrictor FR-902

There are two flow restrictors in the system, each generating a backpressure of 0.2 MPa. One is connected to port 5 on the injection valve to prevent buffer drainage when the injection valve is in position WASTE.

The second flow restrictor is connected after the conductivity cell. It prevents air bubbles being formed after the column when the pressure created in the column is released. Compare to a cork in a bottle of champagne.



This must be allowed for when setting the maximum pressure limit in the methods. For example, when using a column with a pressure limit of 0.3 MPa, the pressure limit in the method should be set to 0.5 MPa. This is described below in detail.

Two types of pressure limits

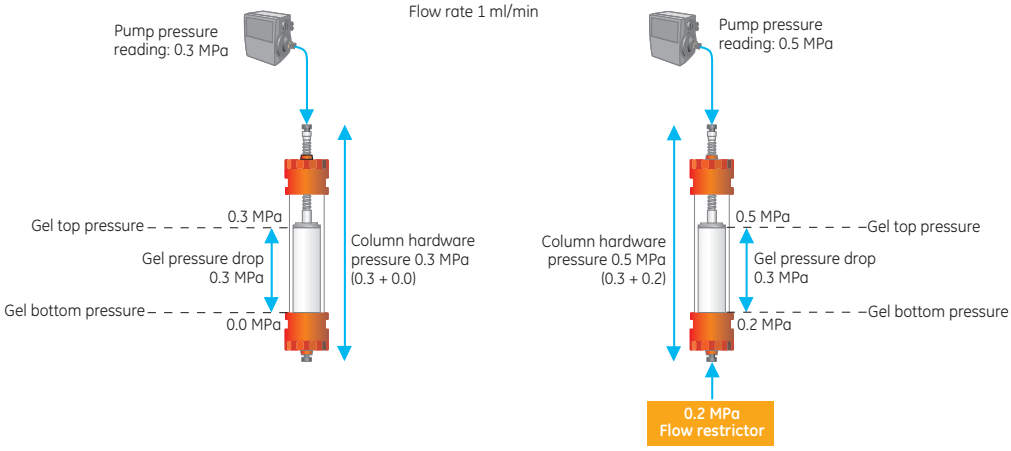
All chromatography columns have two types of pressure limits, one for the column hardware and one limit for the gel packing.

The pressure affecting the column hardware depends on the backpressure generated after the column by, for example flow cells, flow restrictors and tubing. When the pressure limit for the column hardware is exceeded, the column will start leaking. Empty chromatography columns from GE Healthcare are all supplied with data on the column hardware pressure limit.

The pressure affecting the gel packing is dependent on the flow rate and viscosity of the buffer. When the flow rate is too high and/or a high viscosity buffer is used, the pressure limit for the packed gel bed can be exceeded (the gel pressure limit is the maximum allowable pressure drop over the packed gel bed). When the pressure limit is exceeded, the gel particles are forced to the bottom of the column and cause the backpressure to increase. This leads to a collapse of the packed bed and the risk of poor chromatographic performance. The pressure limit data referring to the gel packing is found in the column instructions delivered with all prepacked columns from GE Healthcare.

How the flow restrictor affects the pressure limits

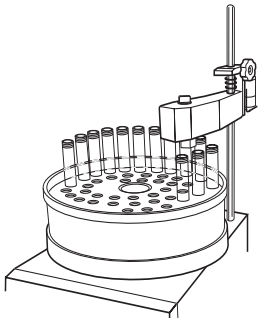
The flow restrictor only affects the column hardware pressure limit. The gel packing pressure limit is always unaffected by a flow restrictor. The figure below shows an example of how a flow restrictor affects the packed column at a hypothetical flow rate of 1 ml/min. The flow rate generates a pump pressure reading of 0.3 MPa. With no restrictor mounted, this pressure equals the pressure drop over both the gel and the column hardware.


Flow rate 1 ml/min
Pump pressure reading: 0.3 MPa
Gel top pressure
0.3 MPa
Gel pressure drop 0.3 MPa
Gel bottom pressure
0.0 MPa
Column hardware pressure 0.3 MPa (0.3 + 0.0)
Pump pressure reading: 0.5 MPa
Column hardware pressure 0.5 MPa (0.3 + 0.2)
0.5 MPa
Gel top pressure
Gel pressure drop 0.3 MPa
Gel bottom pressure
0.2 MPa
0.2 MPa Flow restrictor

When a flow restrictor generating a backpressure of 0.2 MPa is mounted after the column and the same flow rate is used, the pressure over the column hardware is affected and will be 0.5 MPa. Hence, the pump pressure gauge reading will be 0.5 MPa. However, the pressure drop over the packed gel bed is still 0.3 MPa.

Fraction collector

The fraction collector can be used for both small scale and preparative scale purifications. It collects up to 175 fractions in 12 mm diameter tubes and up to 95 fractions in 10–18 mm diameter tubes.



The fraction collector allows fixed volume fractionation or automatic peak fractionation. Fraction marks make it easy to identify fractions and peaks.

Fast tube change minimizes spills between tubes, eliminating it entirely below flow rates of 5 ml/min. Drop synchronization eliminates sample loss during tube change.

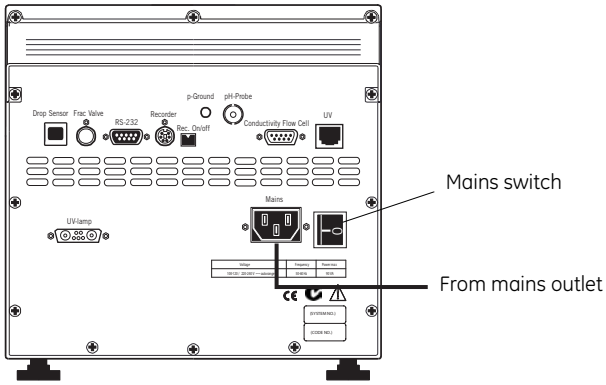
The flow diversion valve mounted on the fraction collector is used as follows:

- Port IN
  Connected to the flow restrictor.
- Port NO (normally open)
  Connected to waste.
- Port NC (normally closed)
  Connected to the tubing holder fitted on the delivery arm on the fraction collector.

11.1.2 Electrical connections

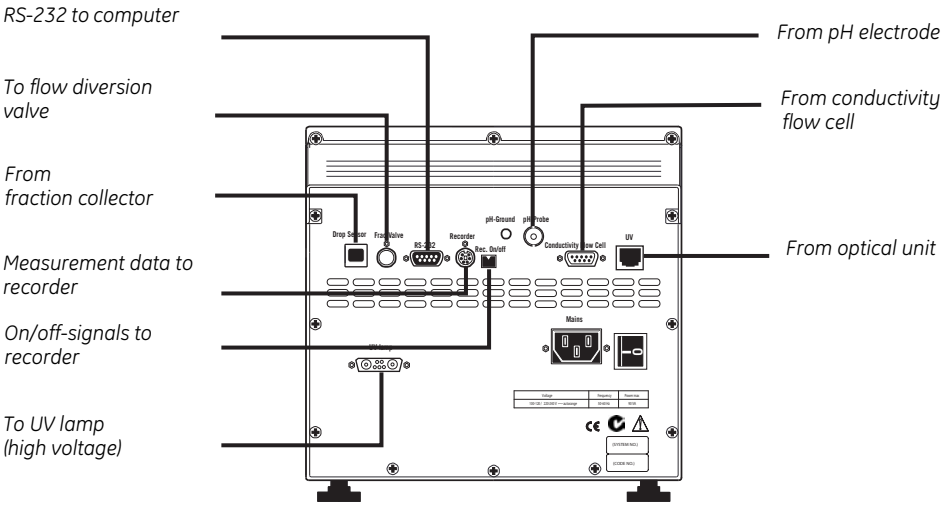
All electrical connections for ÄKTAprime plus are located at the rear of the system.

Mains cable



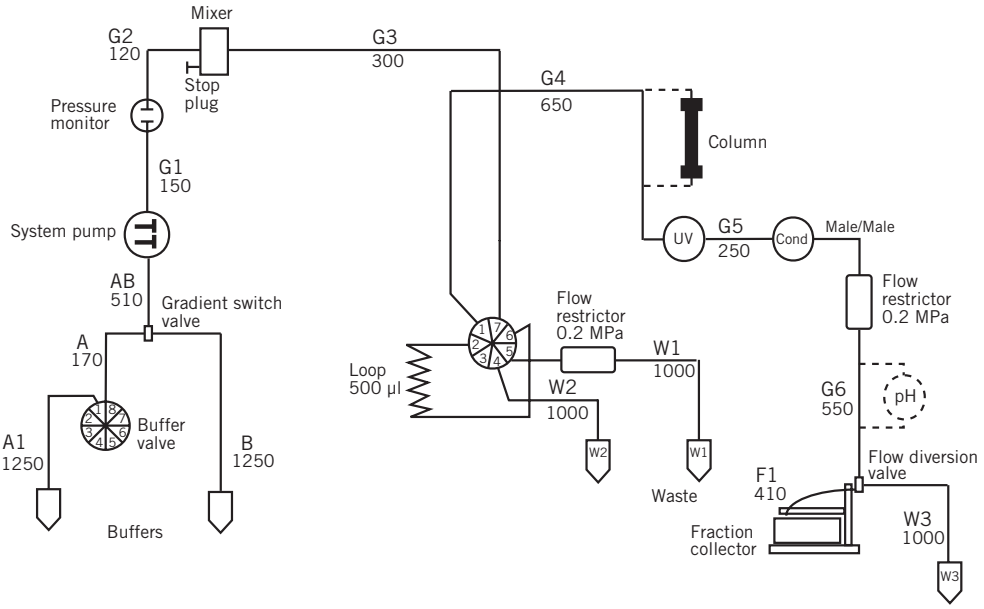
One mains input is required for the system.

Communication cables



11.1.3 Fluid handling path

The following flow diagram shows the positions of the components in ÄKTAprime plus fluid handling path.



All capillaries are fitted at the factory. The figure states the length in millimeters of the pre-fabricated capillaries.

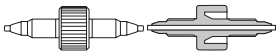
Tubing

The table below lists the tubing fitted at delivery of ÄKTAprime plus.

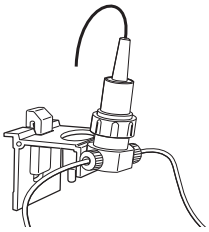
Tubing designation	Tubing i.d. , o.d.	Material	Color	Max. pressure	Volume of 10 cm	Connected
A1, A, B	3.2 mm, 3/16"	Teflon	Clear	3.4 MPa	660.5 µl	All inlet tubings from buffer vessels to switch valve.
AB	1.6 mm, 1/8"	Teflon	Clear	2 MPa	201.1 µl	From switch valve to pump.
W1–W3	1.0 mm, 1/16"	PEEK	Brown	4.1 MPa	78.5 µl	Waste tubing.
G1–G6, F1	0.75 mm, 1/16"	PEEK	Green	2.6 MPa	44.2 µl	From pump to fraction collector.
Union, male/male	i.d. 1/16"	PEEK	Black (orange tubing)	25 MPa	–	Between conductivity flow cell and flow restrictor.
Stop plug	i.d. 1/16"	PEEK	Black	–	–	Mixer inlet.

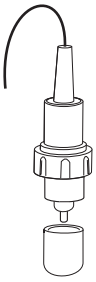
Tubing connectors

The table below lists the connectors and unions used.

Tubing designation	Connector
A1, A, B	Nut and ferrule for o.d. 3/16" tubing (blue ferrule)
AB	Nut and ferrule for o.d. 1/8" tubing (yellow ferrule)
W1–W3, G1–G6, F1	Fingertight connector, for o.d. 1/16" tubing.
Union male/male	Union, 1/16" male/ 1/16" male 
Stop plug	Stop plug, 1/16",

11.1.4 Optional components

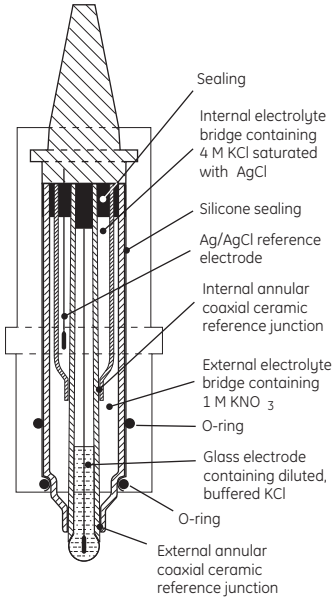




pH flow cell with electrode

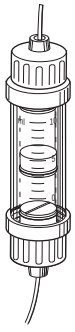
The pH electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1 M KNO_3, an annular ceramic reference junction and a low profile pH membrane. The pH electrode is delivered in a transparent cover.

The flow cell is made of titanium. It should not be used with any other pH electrode.


Sealing
Internal electrolyte bridge containing 4 M KCl saturated with AgCl
Silicone sealing
Ag/AgCl reference electrode
Internal annular coaxial ceramic reference junction
External electrolyte bridge containing 1 M KNO 3
O-ring
Glass electrode containing diluted, buffered KCl
O-ring
External annular coaxial ceramic reference junction

Superloop

Superloop allows introduction of larger sample volumes into a pressurized fluid system. It is used together with the injection valve and replaces a simple sample loop.



Superloop consists of a movable seal in a glass tube. The seal divides the tube into two separate chambers. Depending on the flow direction, the seal moves towards either end piece of the glass tube. Superloop is available in three sizes (10, 50 and 150 ml) allowing application of 1–10, 1–50 and 1–150 ml samples respectively.

11.2 Menus

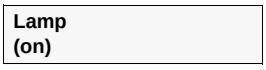
This section describes the Set parameters and Check menus. It also contains an complete overview of the menus in ÄKTAprime plus.

11.2.1 Set Parameters menus

Turning the lamp on/off

We recommend the lamp be turned off to conserve lamp operating time when no measurement is being made. A warm-up time of 60 minutes is required to achieve full specifications. However, in most cases, a warm-up time of 15 minutes is sufficient.

1 Select main menu **Set Parameters** and press **OK**.



2 Select sub menu **Lamp** and press **OK** to access the setting menu.

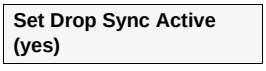
3 Switch the lamp on/off with the up and down buttons, and then press **OK**.

Setting drop synchronization

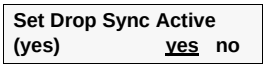
If drop synchronization is active, tube changes will only occur directly after a drop is registered by the drop counter to minimize spillage between tubes. Drop synchronization operates in all fraction collection modes; time, volume and drop.

Drop synchronization is only possible at flow rates up to 3 ml/min. If the flow limit is exceeded, an error message is displayed.

1 Select main menu **Set Parameters** and press **OK**.



2 Select sub menu **Set Drop Sync Active.** The current setting is shown. The setting will apply to all subsequent manual and method controlled operation until a new value is set.
Press **OK** to change the setting.



3 Select the desired setting and press **OK**.

Memory print out

Measurement data from the last run can be printed to a recorder or a computer. Three channels are available for printing the curves that correspond to UV-absorbance, pH, conductivity, concentration of the B-buffer, temperature and pressure.

1 Select main menu **Set Parameters** and press **OK**.

2 Select sub menu **Memory Print Out** and press **OK**.

Print out to? **Recorder Computer**

3 Select printing to a recorder or a computer and press **OK**.

Set Rec Out 1 (UV)

4 The current setting for channel 1 is displayed. Press **OK** to access the setting menu.

Set Rec Out 1 (UV) **UV pH Cond %B Tmp Pr**

5 Select the parameter to be printed on channel 1 and press **OK**.

6 Repeat steps 4 and 5 for channels 2 and 3.

Autoscale UV (no)

7 If the UV-curve is selected, the current setting for auto-scaling of the UV-curve is shown. Press **OK** to access the setting menu.

Autoscale UV (no) **yes no**

8 Select the desired setting and press **OK**.

The three selected curves are now printed.

Setting analog outputs
See section 8.6.5 Setting analog outputs.

Calibrating the flow rate
See section 9.20.1 Calibrating the pump.

Calibrating the pressure offset
See section 9.20.2 Calibrating the pressure sensor.

Set up show pH
Normally, the pH is displayed in the running window. If not required, the pH display can be set to off.

Set Parameters

1 From the main menu, select sub menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration**. Press **OK**.

Setup pH

3 Select sub menu **Setup pH** and press **OK**.

Set Show pH (on)

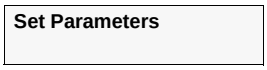
4 Select sub menu **Set Show pH**. The current setting for showing pH is displayed. If **on** is shown, current pH is displayed in the running display. If **off** is shown, no pH is displayed in the running display. Press **OK** to change the setting.

Set Show pH (off) on off

5 Select the desired setting and press **OK**.

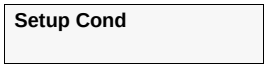
Set up pH calibration
Set up pH temperature compensation
See section *9.20.5 Calibrating the pH electrode (optional).*

Set up show conductivity



1 From the main menu, select menu **Set Parameters** and press **OK**.

2 Select sub menu **Setup and calibration** and press **OK**.



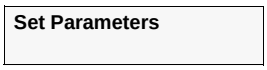
3 Select sub menu **Setup Cond** and press **OK**.

4 Select sub menu **Set Show Cond**. The current status for showing conductivity is shown. If **on** is shown, current conductivity is displayed in the running display. If **off** is shown, no conductivity is displayed in the running display. Press **OK** to change the setting.

5 Change the setting as desired and press **OK**.

Setting the conductivity cell constant
Calibrating the conductivity
Setting the conductivity temperature compensation
Setting the conductivity reference temperature
See section *9.20.3 Calibrating conductivity*.

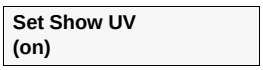
Set up show UV
Normally, UV absorbance is shown in the running display. If not required, the UV absorbance display can be set to off.



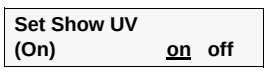
1 From the main menu, select menu **Set Parameters** and press **OK**.

2 Select sub menu **Setup and calibration** and press **OK**.

3 Select sub menu **Setup UV** and press **OK**.



4 Select sub menu **Set Show UV.** The current status for showing UV is shown. If **on** is shown, current UV is displayed in the running display. If **off** is shown, no conductivity is displayed in the running display.
Press **OK** to change the setting.



5 Change the setting as desired and press **OK**.

Set up UV averaging filter constant

To filter the noise in the UV-signal, a moving average filter is used. The averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but it will also distort the peaks. Peaks narrower than the minimum peak width value according to the table below may be distorted. Because of this, the averaging time should be as short as possible. On delivery, the averaging time is set to 1.3 s.

Set Parameters

1 From the main menu, select menu **Set Parameters** and press **OK**.

Setup and calibration

2 Select sub menu **Setup and calibration** and press **OK**.

Setup UV

3 Select sub menu **Setup UV** and press **OK**.

Set Averaging (1.3 s)

4 Select sub menu **Set Averaging**. The current set averaging time is shown. Press **OK** to change the setting.

Set Averaging (1.3 s) **0.64**

5 Set the desired value and press **OK**. Values allowed are 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.3, 2.6, 5 and 10 s.

Averaging time [s]	Corresponding time constant [s] (approximately)	Min. peak width at half height [s]
10.0	5	50
5.1	2	32
2.6	1	16
1.3	0.5	8.0
0.64	0.2	3.2
0.32	0.1	1.6
0.16	0.05	0.8
0.08	0.03	0.5
0.04	0.01	0.2
0.02	0.01	0.1

Set up lamp run time

When the UV lamp is replaced, reset the **Lamp Run Time** counter.

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup UV	3 Select sub menu **Setup UV** and press **OK**.
Set Lamp Run Time Hg (2000 h)	4 Select sub menu **Set Lamp Run Time** press **OK**.
Set Lamp Run Time Hg (2000 h) 000	5 Set the **Lamp Run Time** counter to zero with the dial. Press **OK** to acknowledge.

Set up show temperature

The display of the temperature in the conductivity flow cell, shown in the running display, can be enabled or disabled.

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Setup Temp	3 Select sub menu **Setup Temp** and press **OK**.
Set Show Temp (on)	4 Select sub menu **Set Show Temp**. The current status for showing temperature is displayed. If **on** is shown, current temperature is displayed in the running display. If **off** is shown, no temperature is displayed in the running display. Press **OK** to change the setting.
Set Show Temp (on) on off	5 Change the setting as desired and press **OK**.

Set up Delay UV to Frac

The delay volume (in µl) is the volume of the flow path between the UV flow cell and the fraction collector. If the set delay volume is correct, the event marks on the UV absorbance curve will be synchronized with the tube changes.

Calculate the volume of the tubing from the UV flow cell to the very end of the tubing. The volume of 0.75 mm i.d. tubing is 44.2 µl/10 cm.

The default delay volume is 380 µl.

If using a pH electrode, 100 µl should be added to the delay volume (480 µl) provided that the tubing lengths remain unchanged.

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Set Delay UV to Frac (380 µl)	3 Select sub menu **Set Delay UV to Frac**. The current delay volume is shown. Press **OK** to change the volume.
Set Delay UV to Frac (380 µl) 380	4 Change the setting as desired and press **OK**.

Set up mixer chamber volume

If the mixer chamber is exchanged for a chamber with another volume, the new chamber volume must be set. On delivery, the chamber volume is set to 2.0 ml. If a larger chamber volume is used, the wash and priming volumes must be increased accordingly.

Set Parameters	1 From the main menu, select menu **Set Parameters** and press **OK**.
Setup and calibration	2 Select sub menu **Setup and calibration** and press **OK**.
Set Mix Chamber Vol (2 ml)	3 Select sub menu **Set Mix Chamber Vol**. The current chamber volume is shown. Press **OK** to change the volume.
Set Mix Chamber Vol (2 ml) 2.0	4 Change the setting as desired and press **OK**. Possible values are 0.6, 2.0, 5.0 and 12.0 ml.

11.2.2 Check menus

Checking communication

The communication between the ÄKTAprime plus system and a computer can be checked.

1 Select menu **Check** and press **OK**.

2 The system immediately checks the communication with the computer.

Check Communication **PC App. Connected!**

PC App. Connected! The communication is OK.

Check Communication **PC Driver Connected!**

PC Driver Connected! PrimeView software is not properly installed.

Check Communication **Not Connected!**

Not Connected The serial cable between the system and the computer is not properly connected , or PrimeView software is not installed on the computer.

Checking autozero level

The module internal absorbance value for autozero can be checked to test the consistency of buffers.

Check Autozero **AZ 0.00006AU**

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Autozero**. The autozero absorbance value for the used wavelength is shown.

Checking lamp run time

The lamp run time can be checked to determine the need for lamp replacement. Run times for both Hg and Zn lamps are monitored.

Check Lamp Run Time **Hg 1482h Zn 430h**

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Lamp Run Time**.

Checking lamp intensity

The lamp intensity can be checked to determine the status of the lamp used.

Check Lamp Intensity **R 215,5 S 214.4mV**

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Lamp Intensity**.

Checking pump run time

The pump run time can be checked to determine the need for maintenance.

Check Pump Run Time
246h

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Pump Run Time**.

Checking pumped volume

The volume delivered by the pump can be checked to determine the need for maintenance.

Check Pumped Volume
3567 ml

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Pumped Volume**.

Checking tube shifts

The number of tube shifts done by the fraction collector can be checked to determine the need for maintenance.

Check Tube Shifts
3592

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Tube Shifts**.

Checking valve shifts

The number of shifts done by the buffer valve and the injection valve can be checked to determine the need for maintenance.

Check Valve Shifts
BV: 642 IV: 348

1 Select menu **Check** and press **OK**.

2 Select sub menu **Check Valve Shifts**.

Checking the recorder

The function of a connected chart recorder can be tested.

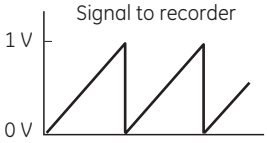
1 Select menu **Check** and press **OK**.

Check Recorder

2 Select sub menu **Check Recorder**.

3 Press **OK** to start the test.

The test will ramp the signal on each channel up to 1 V and then immediately decrease the signal back to 0 V. The test is run continuously. Compare the diagram of the chart recorder with the figure.



4 Stop the test by pressing **OK** or **Esc**.

Checking service mode

Service information relevant to the module can be checked. Information may not be available in all menus.

1 Select main menu **Check** and press **OK**.

Check Service Mode

2 Select menu **Check Service Mode** and press **OK**.

Telephone Service: **012345678901**

3 The service telephone number is displayed. Press **OK**.

Contract number: **01234567801**

4 The service contract number is displayed. Press **OK**.

Serial Number: **0123456 YM 012345**

5 The module serial number is displayed. Press **OK**.

ÄKTAprime **V3.00**

6 The system name and software version are displayed. Press **OK**.

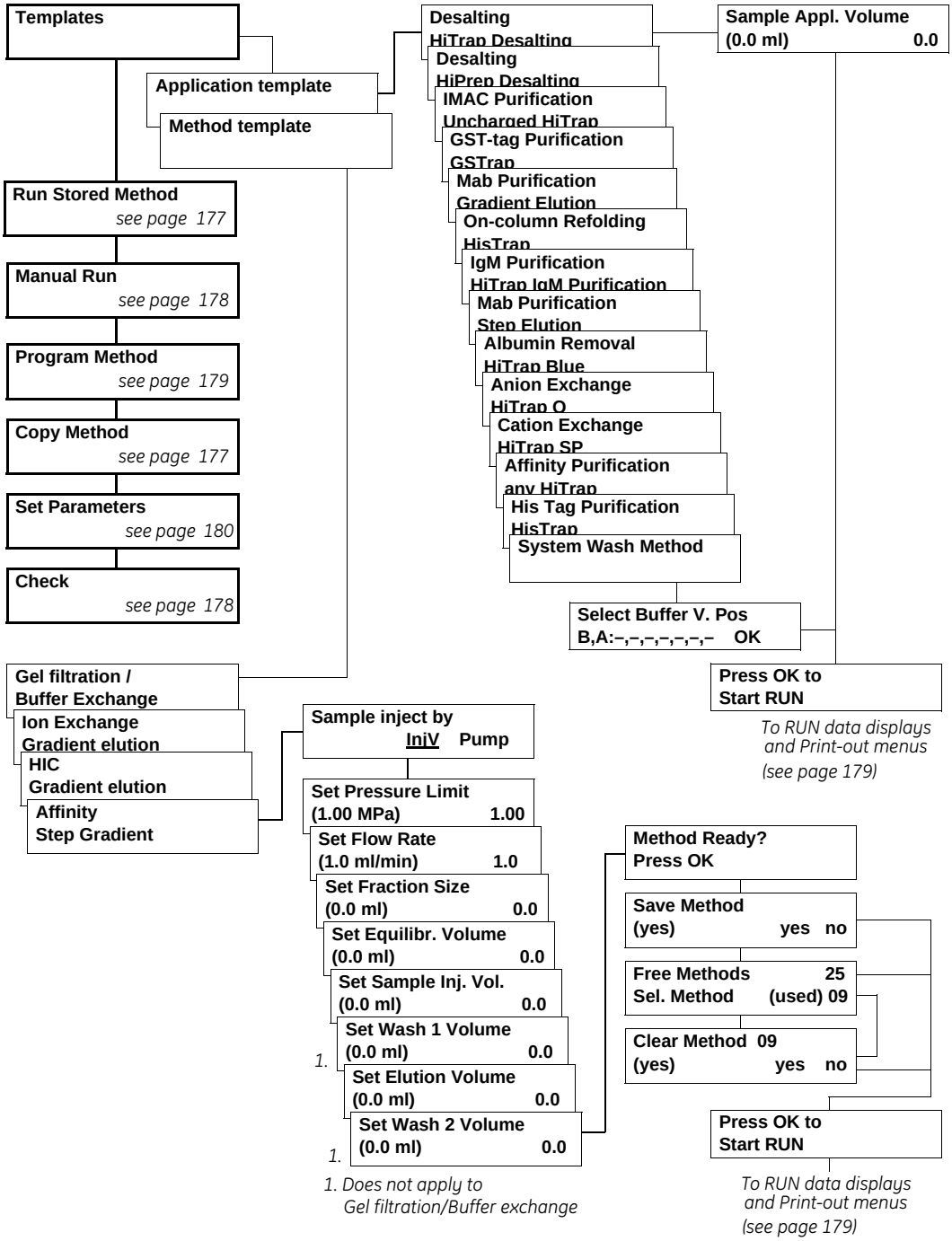
Date of maintenance: **DD MMMM YYYY**

7 The date of the last service is displayed. Press **OK**.

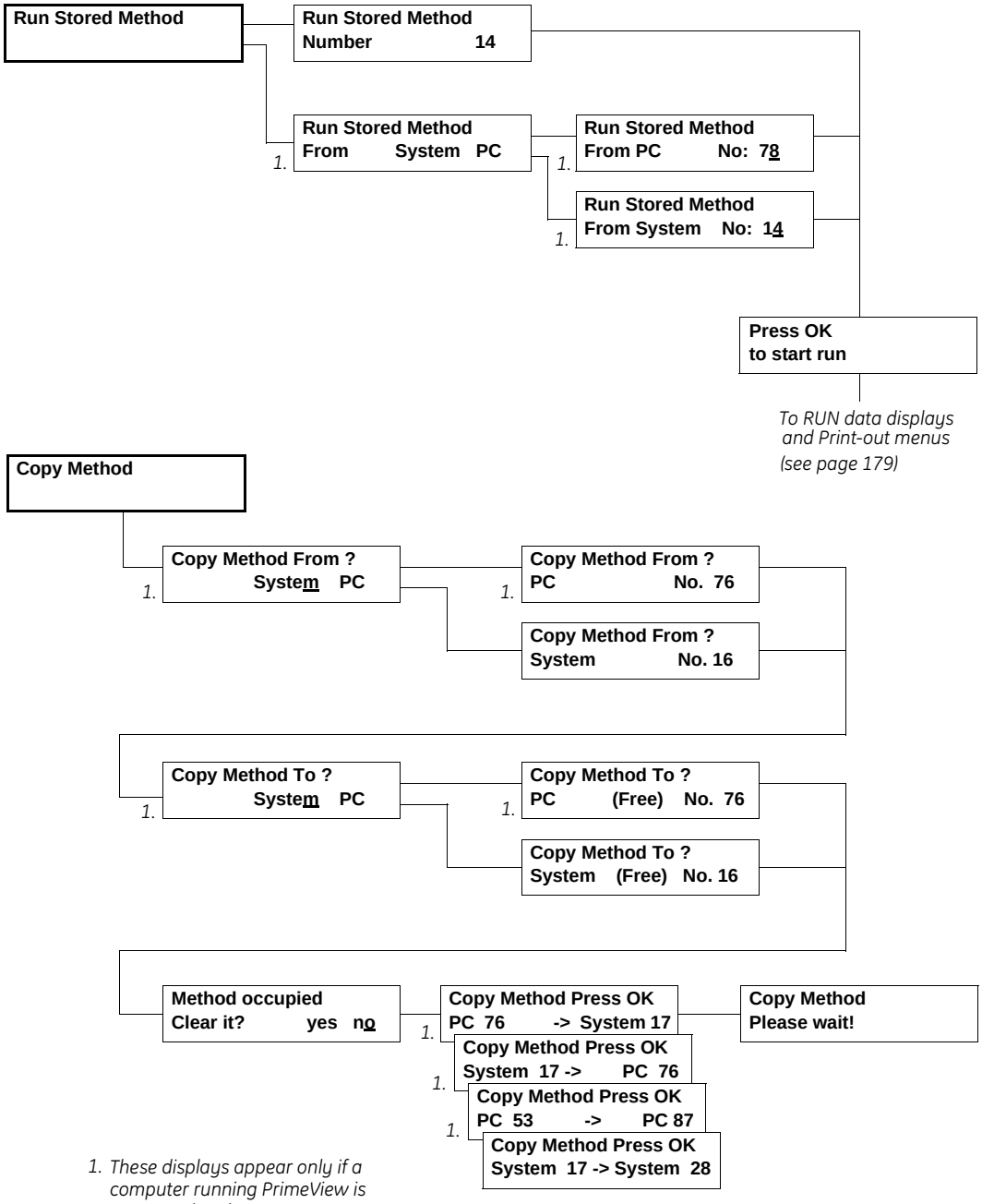
Buzzer Test

8 A test of the system buzzer is performed. Press **OK**.

11.2.3 Menu overview

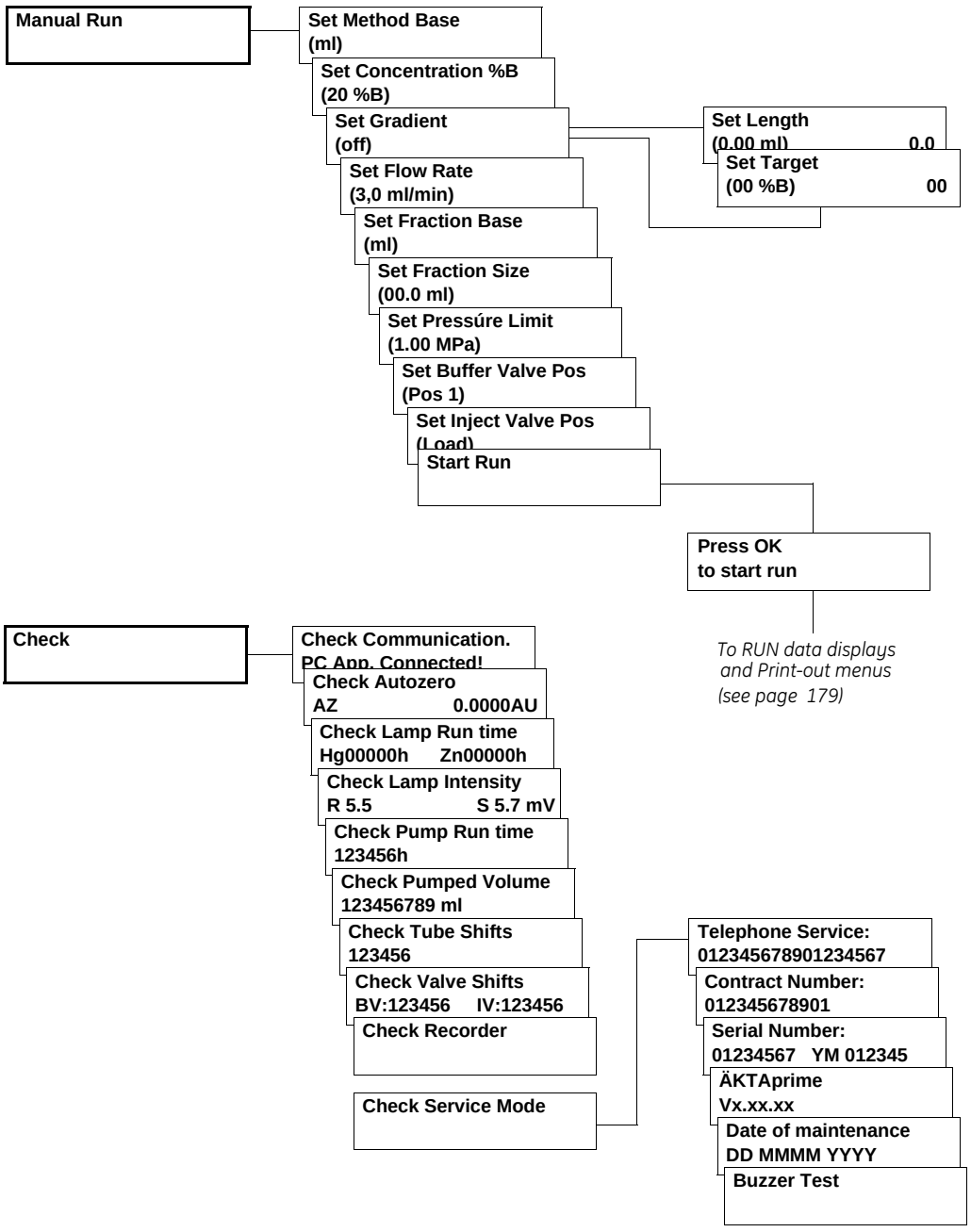


Menu overview (cont.) 2

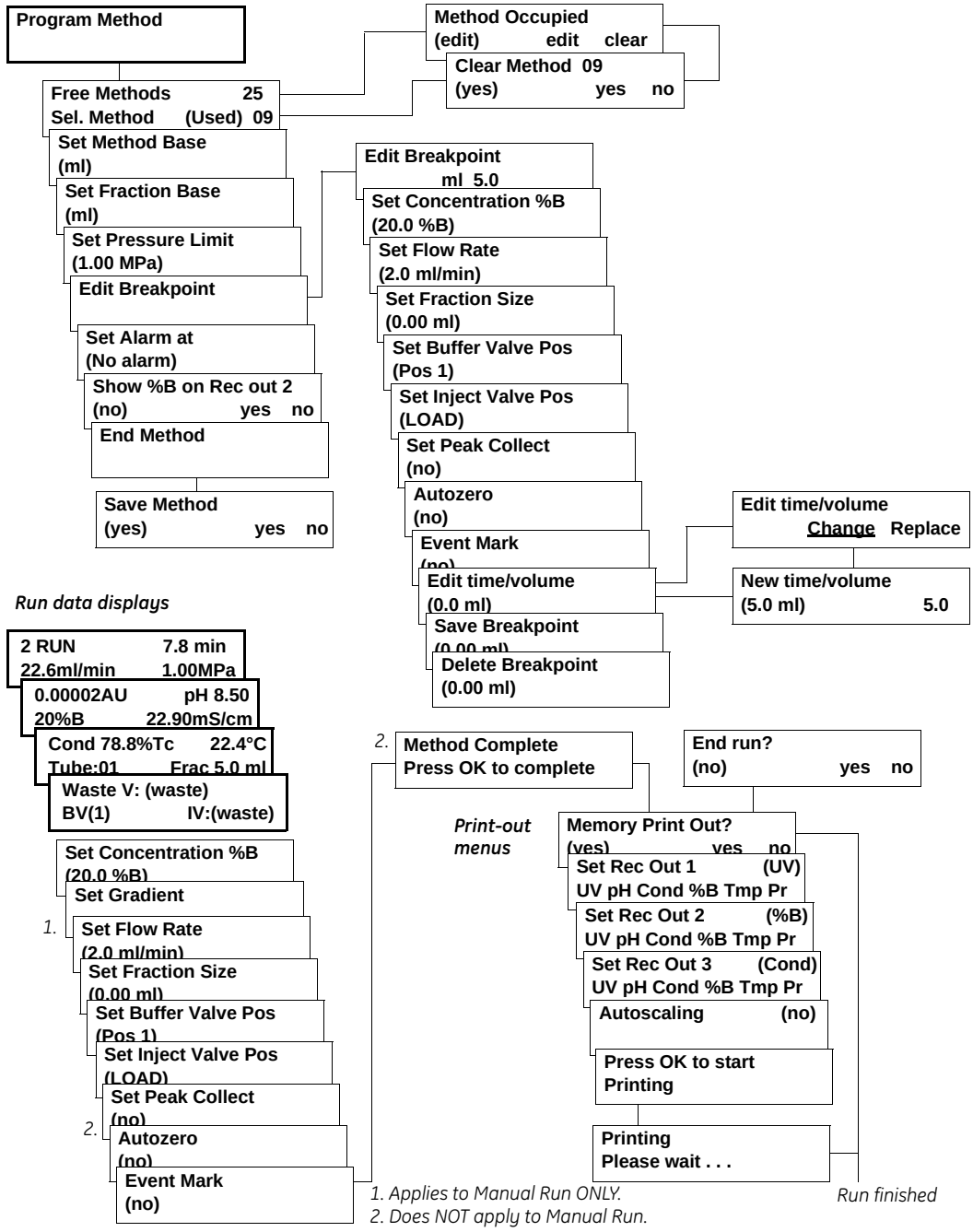

Run Stored Method
Run Stored Method Number 14
Run Stored Method From System PC
Run Stored Method From PC No: 78
Run Stored Method From System No: 14
Press OK to start run
To RUN data displays and Print-out menus (see page 179)
Copy Method
Copy Method From ? System PC
Copy Method From ? PC No. 76
Copy Method From ? System No. 16
Copy Method To ? System PC
Copy Method To ? PC (Free) No. 76
Copy Method To ? System (Free) No. 16
Method occupied Clear it? yes no
Copy Method Press OK PC 76 -> System 17
Copy Method Press OK System 17 -> PC 76
Copy Method Press OK PC 53 -> PC 87
Copy Method Press OK System 17 -> System 28
Copy Method Please wait!
1.

1. *These displays appear only if a computer running PrimeView is connected to the system.*

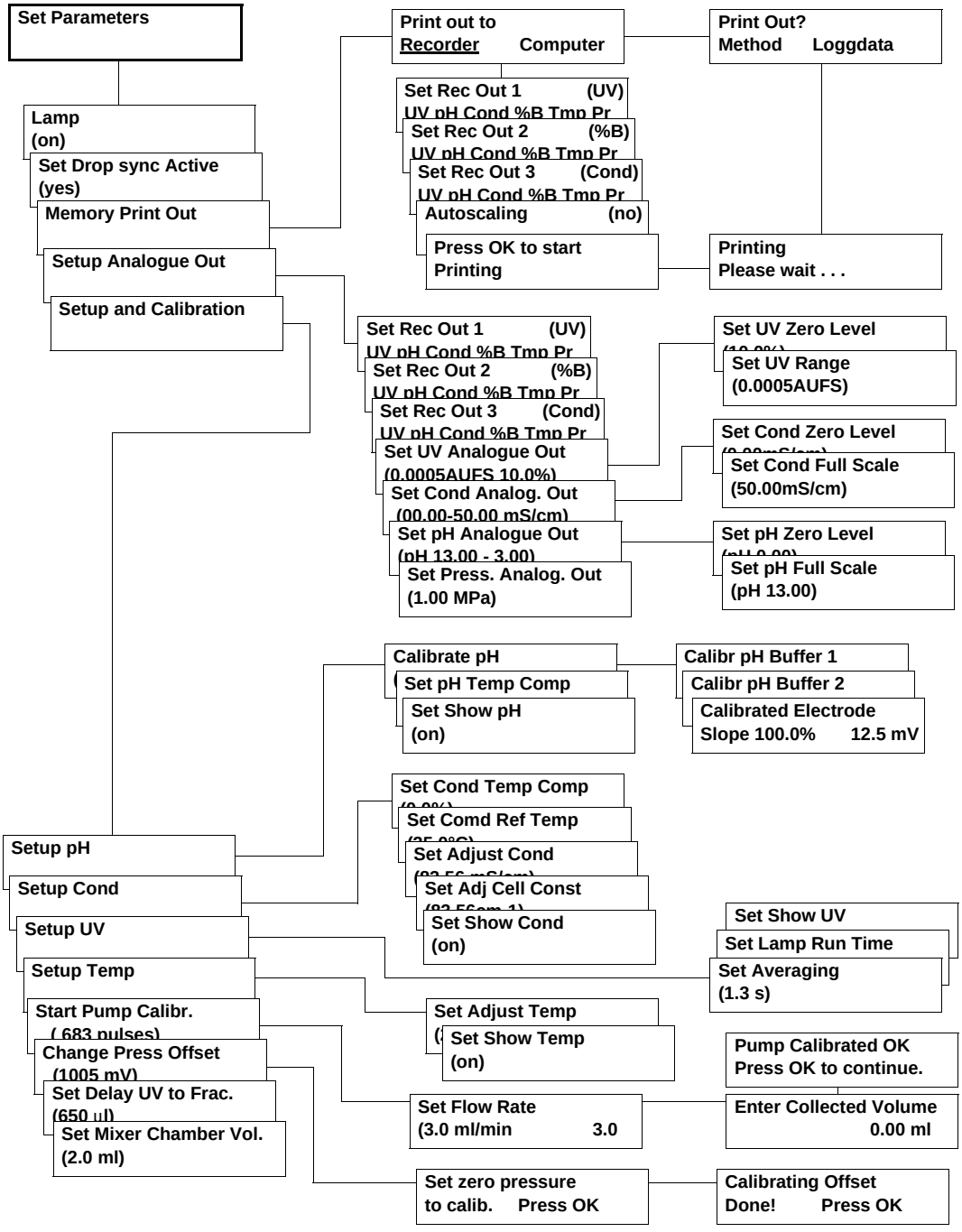
Menu overview (cont.) 3


Manual Run
Set Method Base (ml)
Set Concentration %B (20 %B)
Set Gradient (off)
Set Length (0.00 ml) 0.0
Set Target (00 %B) 00
Set Flow Rate (3,0 ml/min)
Set Fraction Base (ml)
Set Fraction Size (00.0 ml)
Set Pressúre Limit (1.00 MPa)
Set Buffer Valve Pos (Pos 1)
Set Inject Valve Pos (Load)
Start Run
Press OK to start run
To RUN data displays and Print-out menus (see page 179)
Check
Check Communication. PC App. Connected!
Check Autozero AZ 0.0000AU
Check Lamp Run time Hg00000h Zn00000h
Check Lamp Intensity R 5.5 S 5.7 mV
Check Pump Run time 123456h
Check Pumped Volume 123456789 ml
Check Tube Shifts 123456
Check Valve Shifts BV:123456 IV:123456
Check Recorder
Check Service Mode
Telephone Service: 012345678901234567
Contract Number: 012345678901
Serial Number: 01234567 YM 012345
ÄKTAprime Vx.xx.xx
Date of maintenance DD MMMM YYYY
Buzzer Test

Menu overview (cont.) 4



Menu overview (cont.) 5


Set Parameters
Lamp (on)
Set Drop sync Active (yes)
Memory Print Out
Setup Analogue Out
Setup and Calibration
Print out to Recorder Computer
Print Out? Method Loggdata
Set Rec Out 1 (UV) UV pH Cond %B Tmp Pr
Set Rec Out 2 (%B) UV pH Cond %B Tmp Pr
Set Rec Out 3 (Cond) UV pH Cond %B Tmp Pr
Autoscaling (no)
Press OK to start Printing
Printing Please wait . . .
Set Rec Out 1 (UV) UV pH Cond %B Tmp Pr
Set Rec Out 2 (%B) UV pH Cond %B Tmp Pr
Set Rec Out 3 (Cond) UV pH Cond %B Tmp Pr
Set UV Analogue Out (0.0005AUFS 10.0%)
Set Cond Analog. Out (00.00-50.00 mS/cm)
Set pH Analogue Out (pH 13.00 - 3.00)
Set Press. Analog. Out (1.00 MPa)
Set UV Zero Level
Set UV Range (0.0005AUFS)
Set Cond Zero Level
Set Cond Full Scale (50.00mS/cm)
Set pH Zero Level
Set pH Full Scale (pH 13.00)
Calibrate pH
Set pH Temp Comp
Set Show pH (on)
Calibr pH Buffer 1
Calibr pH Buffer 2
Calibrated Electrode Slope 100.0% 12.5 mV
Set Cond Temp Comp
Set Comd Ref Temp
Set Adjust Cond
Set Adj Cell Const
Set Show Cond (on)
Setup pH
Setup Cond
Setup UV
Setup Temp
Start Pump Calibr. (683 pulses)
Change Press Offset (1005 mV)
Set Delay UV to Frac. (650 µl)
Set Mixer Chamber Vol. (2.0 ml)
Set Adjust Temp
Set Show Temp (on)
Set Flow Rate (3.0 ml/min 3.0
Set zero pressure to calib. Press OK
Set Show UV
Set Lamp Run Time
Set Averaging (1.3 s)
Pump Calibrated OK Press OK to continue.
Enter Collected Volume 0.00 ml
Calibrating Offset Done! Press OK

11.3 Technical specifications

Relevant system and component specifications are listed below.

11.3.1 Operating data

Pump	
Flow rate range	0.1–50 ml/min in steps of 0.1 ml/min
Operating pressure range	0–1.0 MPa (10 bar, 145 psi)
Pressure pulsation	≤ ±10% of mean value in entire range
pH stability range	1–14 (spec. valid between pH 2–12)
Viscosity *≤10 ml/min* *> 10 ml/min*	 Max. 10 cP Max. 5 cP
Flow rate reproducibility	rsd ≤ 1% during one day (pressure ≥ 0.1 MPa, flow rate ≥ 0.4 ml/min)
Flow rate accuracy	-5%– +3% of setting (pressure ≥ 0.1 MPa, flow rate ≥ 0.4 ml/min)
Gradient composition *accuracy* *reproducibility*	 ±3% at 0.1–50 ml/min (5–95%B) ±1.0% at 0.1–50 ml/min (5–95%B)
Leakage	< 1.0 µl/min (complete system)
Pressure sensor *range* *scale error* *offset error*	 0–1.1 MPa ±2% ±0.02 MPa
UV measurement	
Wavelengths *Hg lamp, fixed* *by changing filter (option)* *Zn lamp (optional)*	 254 and 280 nm 313, 365, 405, 436 and 546 nm 214 nm
Absorbance range	0.001–5.0 AU
Autozero range	-0.2–2.0 AU
Baseline adjust	Adjustable 0–100% of full scale
Linearity	< 3% up to 2 AU at 254 nm < 5% at other wavelengths except 280 nm
Static noise *short term* *long term*	 40×10^{-6} AU at 254 nm 40×10^{-6} AU at 254 nm
Static drift	$\pm 100 \times 10^{-6}$ AU/hour at 254 nm
Flow sensitivity	2×10^{-4} AU min/ml

UV flow cell, 2 mm	
Flow rate	0–100 ml/min
Max. pressure	4.0 MPa
Max. backpressure	0.05 MPa at 100 ml/min
Liquid temperature range	+4 to +60 °C
Optical path length	2 mm
Cell volume	2 µl (30 µl detector volume)
UV flow cell, 5 mm (optional)	
Flow rate	0–20 ml/min
Max. pressure	4.0 MPa
Max. backpressure	0.02 MPa at 20 ml/min
Optical path length	5 mm
Cell volume	6 µl (10 µl detector volume)
Conductivity measurement	
Conductivity range	1 µS/cm to 999.9 mS/cm
Reproducibility *short term* *long term*	 Max. ±1% or ±5 µS/cm whichever is greater Max. ±3% or ±15 µS/cm whichever is greater
Noise	Max. ±0.5% of full scale calibrated range
Response time	< 3 s (0–95% of step)
Temperature sensor *accuracy* *drift*	 ±2.0 °C ±0.5 °C per 10 h
Flow rate sensitivity	±1% within 0–100 ml/min
Conductivity flow cell	
Flow rate	0–100 ml/min
Max. pressure	5 MPa (50 bar, 725 psi)
Max. backpressure	0.01 MPa at 100 ml/min
Fraction collection	
Tube capacity	95 in tube rack 18 mm 175 in tube rack 12 mm (optional)

pH measurement	
pH range	0 to 14 (spec. valid between 2 and 12)
Accuracy *temperature compensated* *not compensated*	 ±0.1 pH within +4 to +40 °C ±0.2 pH within +15 to 25 °C, ±0.5 pH within +4 to +15 °C and +25 to +40 °C
Response time	< 10 s (0–95% of step)
Long term stability	Dev. max. 0.1 pH per 10 h at constant conditions (4–40 °C)
Flow rate sensitivity	Dev. max. 0.1 pH units
pH cell	
Flow rate	0.1–100 ml/min
Max. pressure	0.5 MPa (5 bar, 72 psi)
Max. backpressure	0.02 MPa at 100 ml/min

11.3.2 Physical data

Control	Via membrane keyboard and display (2x20 characters)
Degree of protection *housing* *flow cells*	 IP 20 IP 44
Power requirement	100–120/220–240 V ~ autorange, 50–60 Hz
Power consumption	Max. 90 VA
Fuse specification	T 1.0 AH/250 VAC, approved type (not replaceable by operator)
Dimensions, H x W x D	530 x 400 x 450 mm
Weight	13 kg
Environment	+4 to +40 °C, 10–95% relative humidity (non-condensing), 84–106 kPa (840–1060 mbar atmospheric pressure).
Compliance with standards	The declaration of conformity is valid for the instrument only if it is: • used in laboratory locations • used in the same state as it was delivered from GE Healthcare except for alterations described in the User Manual • connected to other CE labelled GE Healthcare modules or other products as recommended.
Safety standards	This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the following harmonized standards: • EN 61010-1 • IEC 61010-1 • CAN/CSA-C22.2 No. 61010-1 • UL61010-1

EMC standards	This device meets the requirements of the EMC Directive 89/336/EEC through the following harmonized standards: • EN 61326 (emission and immunity) • EN 55011, GR 2, Class A (emission) • This device complies with part 15 of the FCC rules (emission). Operation is subject to the following two conditions: 1 This device may not cause harmful interference. 2 This device must accept any interference received, including interference that may cause undesired operation.

11.3.3 Wetted materials

The wetted materials in ÄKTAprime plus are listed below:

	FFKM	PEEK	PTFE	FEP	ETFE	ECTFE	PP	PE	Titanium alloy	Elgiloy	UHMWPE	Sapphire	Quartz	Glass	Gold	Simriz	Ceramic
Pump		X							X	X	X	X		X		X	X
Monitor	X	X	X						X				X	X			
Fraction collector			X			X											
Mixer	X	X	X														
Pressure sensor																	
Buffer valve/ Injection valve		X															
Switch valve/ Flow diversion valve			X			X											
Flow restrictor		X	X		X										X		
Superloop	X	X			X									X			
Tubing		X		X													
Inlet filters							X		X								
Unions/Connectors		X			X												

FFKM = perfluororubber
PEEK = polyetheretherketone
PTFE = polytetrafluoroethylene
FEP = perfluoroethylenepropylene copolymer
ETFE = ethylenetetrafluoroethylene
ECTFE = ethylenechlorotrifluoroethylene
UHMWPE = Ultra-high molecular weight polyethylene
PE = polyethene
PP = polypropylene

11.4 Chemical resistance guide and chemical compatibility

The chemical resistance of ÄKTAprime plus to some of the most commonly used chemicals in liquid chromatography is indicated in the table below.

The ratings are based on the following assumptions:

1 The synergistic effects of the chemical mixtures have not been taken into account.

2 Room temperature and limited over-pressure is assumed.

Note: *Chemical influences are time and pressure dependent. Unless otherwise stated, all concentrations are 100%.*

Chemical	Exposure	Comments
Acetic acid, 0.1 M	OK	
Acetone, 1%	OK	
Aqueous buffers, pH 2–12	OK	
Decon 90, 10%	OK	For washing only
Ethanol, 20%	OK	
Ethanol, 96%	OK	For washing only
Ethylene glycol	OK	
Formic acid, 1%	OK	
Guanidin, 6 M	OK	
HCl, 0.1 M	OK	
Isopropanol, 30%	OK	
Lysozyme, 2 mg/ml	OK	
Methanol, 20%	OK	
NaOH, 0.1 M	OK	
NaOH, 1 M	OK	For washing only
SDS, 10%	OK	Short term use
TFA, 0.2%	OK	
Triton-X, 2%	OK	Short term use
Urea, 8 M	OK	

11.5 Ordering information

11.5.1 Recommended columns

Ion Exchange Columns

Column name	Pack size	Code no.
HiTrap™ Q HP, 1 ml	5 × 1 ml	17-1153-01
HiTrap Q HP, 5 ml	5 × 5 ml	17-1154-01
HiTrap SP HP, 1 ml	5 × 1 ml	17-1151-01
HiTrap SP HP, 5 ml	5 × 5 ml	17-1152-01
HiTrap IEX Selection kit	7 × 1 ml	17-6002-33
HiTrap Q FF, 1 ml	5 × 1 ml	17-5053-01
HiTrap Q FF, 5 ml	5 × 5 ml	17-5156-01
HiTrap SP FF, 1 ml	5 × 1 ml	17-5054-01
HiTrap SP FF, 5 ml	5 × 5 ml	17-5157-01
HiTrap DEAE FF, 1 ml	5 × 1 ml	17-5055-01
HiTrap DEAE FF, 5 ml	5 × 5 ml	17-5154-01
HiTrap CM FF, 1 ml	5 × 1 ml	17-5056-01
HiTrap CM FF, 5 ml	5 × 5 ml	17-5155-01
HiTrap ANX FF (high sub), 1 ml	5 × 1 ml	17-5162-01
HiTrap ANX FF (high sub), 5 ml	5 × 5 ml	17-5163-01
HiTrap Q XL, 1 ml	5 × 1 ml	17-5158-01
HiTrap Q XL, 5 ml	5 × 5 ml	17-5159-01
HiTrap SP XL, 1 ml	5 × 1 ml	17-5160-01
HiTrap SP XL, 5 ml	5 × 5 ml	17-5161-01
HiPrep™ 16/10 Q XL	1 × 20 ml	17-5092-01
HiPrep 16/10 SP XL	1 × 20 ml	17-5093-01
HiPrep 16/10 CM FF	1 × 20 ml	17-5091-01
HiPrep 16/10 DEAE FF	1 × 20 ml	17-5090-01
HiPrep 16/10 Q FF	1 × 20 ml	17-5190-01
HiPrep 16/10 ANX FF (high sub)	1 × 20 ml	17-5191-01
HiPrep 16/10 SP FF	1 × 20 ml	17-5192-01

Buffer Exchange/Desalting Columns

Column name	Pack size	Code no.
HiTrap Desalting, 5 ml	5 × 5 ml	17-1408-01
HiTrap Desalting, 5 ml	100 × 5 ml[1]	11-0003-29
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

[1] Pack size available on special order.

Size Exclusion (Gel filtration) Columns

Column name	Pack size	Code no.
HiPrep 16/60 Sephacryl™ S-100 HR	1 × 120 ml	17-1165-01
HiPrep 26/60 Sephacryl S-100 HR	1 × 320 ml	17-1194-01
HiPrep 16/60 Sephacryl S-200 HR	1 × 120 ml	17-1166-01
HiPrep 26/60 Sephacryl S-200 HR	1 × 320 ml	17-1195-01
HiPrep 16/60 Sephacryl S-300 HR	1 × 120 ml	17-1167-01
HiPrep 26/60 Sephacryl S-300 HR	1 × 320 ml	17-1196-01

Chelating Columns

Column name	Pack size	Code no.
HiTrap Chelating HP, 1 ml	5 × 1 ml	17-0408-01
HiTrap Chelating HP, 5 ml	1 × 5 ml	17-0409-01
HiTrap Chelating HP, 5 ml	5 × 5 ml	17-0409-03
HiTrap Chelating HP, 5 ml	100 × 5 ml[1]	17-0409-05

[1] Pack size available on special order.

Affinity Columns

Column name	Pack size	Code no.
HiTrap Protein A HP, 1 ml	5 × 1 ml	17-0402-01
HiTrap Protein A HP, 1 ml	2 × 1 ml	17-0402-03
HiTrap Protein A HP, 5 ml	1 × 5 ml	17-0403-01
HiTrap Protein G HP, 1 ml	5 × 1 ml	17-0404-01
HiTrap Protein G HP, 1 ml	2 × 1 ml	17-0404-03
HiTrap Protein G HP, 5 ml	1 × 5 ml	17-0405-01
HiTrap Heparin HP, 1 ml	5 × 1 ml	17-0406-01
HiTrap Heparin HP, 5 ml	1 × 5 ml	17-0407-01
HiPrep 16/10 Heparin FF	1 × 20 ml	17-5189-01
HiTrap rProtein A HP, 1 ml	5 × 1 ml	17-5079-01
HiTrap rProtein A HP, 1 ml	2 × 1 ml	17-5079-02

Column name	Pack size	Code no.
HiTrap rProtein A HP, 5 ml	1 × 5 ml	17-5080-01
HiTrap Blue HP, 1 ml	5 × 1 ml	17-0412-01
HiTrap Blue HP, 5 ml	1 × 5 ml	17-0413-01
HiTrap NHS-activated HP, 1 ml	5 × 1 ml	17-0716-01
HiTrap NHS-activated HP, 5 ml	1 × 5 ml	17-0717-01
HiTrap NHS-activated HP, 1 ml	180 × 1 ml[1]	17-0716-30
HiTrap IgM Purification HP, 1 ml	5 × 1 ml	17-5110-01
HiTrap IgY Purification HP, 5 ml	1 × 5 ml	17-5111-01
HiTrap Streptavidin HP, 1 ml	5 × 1 ml	17-5112-01
HisTrap™ HP, 1 ml	5 × 1 ml	17-5247-01
HisTrap HP, 1 ml	100 × 1 ml[1]	17-5247-05
HisTrap HP, 5 ml	1 × 5 ml	17-5248-01
HisTrap HP, 5 ml	5 × 5 ml	17-5248-02
HisTrap HP, 5 ml	100 × 5 ml[1]	17-5248-05
HisTrap FF, 1 ml	5 × 1 ml	17-5319-01
HisTrap FF, 1 ml	100 × 1 ml[1]	17-5319-02
HisTrap FF, 5 ml	5 × 5 ml	17-5255-01
HisTrap FF, 5 ml	100 × 5 ml[1]	17-5255-02
HisPrep™ FF 16/10	1 × 20 ml	17-5256-01
HisTrap FF crude, 1 ml	5 × 1 ml	11-0004-58
HisTrap FF crude, 1 ml	100 × 1 ml[1]	11-0004-59
HisTrap FF crude, 5 ml	5 × 5 ml	17-5286-01
HisTrap FF crude, 5 ml	100 × 5 ml[1]	17-5286-02
GSTrap™ HP, 1 ml	5 × 1 ml	17-5281-01
GSTrap HP, 1 ml	100 × 1 ml[1]	17-5281-02
GSTrap HP, 5 ml	1 × 5 ml	17-5282-01
GSTrap HP, 5 ml	5 × 5 ml	17-5282-02
GSTrap HP, 5 ml	100 × 5 ml[1]	17-5282-05
GSTrap FF, 1 ml	5 × 1 ml	17-5130-01
GSTrap FF, 1 ml	2 × 1 ml	17-5130-02
GSTrap FF, 1 ml	100 × 1 ml[1]	17-5130-05
GSTrap FF, 5 ml	1 × 5 ml	17-5131-01
GSTrap FF, 5 ml	5 × 5 ml	17-5131-02
GSTrap FF, 5 ml	100 × 5 ml[1]	17-5131-05
GSTPrep™ FF 16/10	1 × 20 ml	17-5234-01
GSTrap FF crude, 1 ml	5 × 1 ml	11-0004-60
GSTrap FF crude, 1 ml	100 × 1 ml[1]	11-0004-61
GSTrap FF crude, 5 ml	5 × 5 ml	17-5284-02

Column name	Pack size	Code no.
GSTrap FF crude, 5 ml	100 × 5 ml[1]	17-5284-03
HiTrap Benzamidine FF (high sub), 1 ml	2 × 1 ml	17-5143-02
HiTrap Benzamidine FF (high sub), 1 ml	5 × 1 ml	17-5143-01
HiTrap Benzamidine FF (high sub), 5 ml	1 × 5 ml	17-5144-01

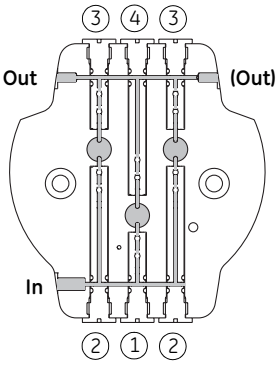
[1] Pack size available on special order.

Hydrophobic Interaction Columns

Column name	Pack size	Code no.
HiTrap HIC Selection kit	5 × 1 ml	17-1349-01
HiTrap Phenyl FF (high sub), 1 ml	5 × 1 ml	17-1355-01
HiTrap Phenyl FF (high sub), 5 ml	5 × 5 ml	17-5193-01
HiTrap Phenyl FF (low sub), 1 ml	5 × 1 ml	17-1353-01
HiTrap Phenyl FF (low sub), 5 ml	5 × 5 ml	17-5194-01
HiTrap Phenyl HP, 1 ml	5 × 1 ml	17-1351-01
HiTrap Phenyl HP, 5 ml	5 × 5 ml	17-5195-01
HiTrap Octyl FF, 1 ml	5 × 1 ml	17-1359-01
HiTrap Octyl FF, 5 ml	5 × 5 ml	17-5196-01
HiTrap Butyl FF, 1 ml	5 × 1 ml	17-1357-01
HiTrap Butyl FF, 5 ml	5 × 5 ml	17-5197-01
HiPrep 16/60 Phenyl FF (high sub)	1 × 20 ml	17-5095-01
HiPrep 16/60 Phenyl FF (low sub)	1 × 20 ml	17-5094-01
HiPrep 16/60 Octyl FF	1 × 20 ml	17-5097-01
HiPrep 16/60 Butyl FF	1 × 20 ml	17-5096-01

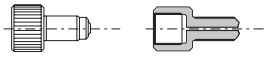
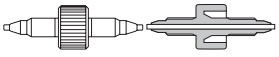
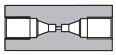
11.5.2 Consumables and accessories

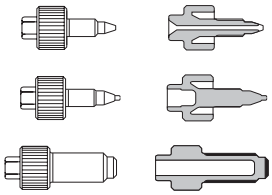
Item	Quant./ pack	A/C*	Code no.
ÄKTAprime plus incl. REC 112	1	C	11-0013-12
ÄKTAprime plus without REC 112	1	C	11-0013-13
ÄKTAprime plus without REC 112 (Japanese version)	1	C	11-0013-14
PrimeView software kit incl. CD, manual and serial cable	1	A	18-1152-84
Pump			
Inlet check valve, short (1)	1	C	18-1172-43
Inlet check valve, long (2)	1	C	18-1172-44
Outlet check valve, short (3)	1	C	18-1172-45
Outlet check valve, long (4)	1	C	18-1172-46
O-ring kit, P-960, incl. 1.42 × 1.52 mm (connection part) 4 × 1.2 mm (check valves)	 7 12	C	18-1172-53
Optical unit			
Hg lamp & housing complete	1	C	18-1128-22
Zn lamp & housing complete	1	C	18-1128-23
UV flow cell 5 mm	1	C	18-1128-24
UV flow cell 2 mm	1	C	18-1128-25
Filter 214 nm	1	C	18-0622-01
Filter 254 nm	1	C	18-0620-01
Filter 280 nm	1	C	18-0621-01
Filter 313 nm	1	C	18-0623-01
Filter 365 nm	1	C	18-0624-01
Filter 405 nm	1	C	18-0625-01
Filter 436 nm	1	C	18-0626-01
Filter 546 nm	1	C	18-0627-01
Filter wheel complete	1	A	18-0647-01
Conductivity flow cell			
Conductivity flow cell	1	C	18-1111-05
pH electrode			
pH electrode, round tip, incl. flow cell and holder	1	C	18-1134-84
pH electrode, round tip	1	C	18-1111-26
pH flow cell, round tip, incl. dummy electrode	1	A	18-1112-92
Dummy electrode, round tip	1	A	18-1111-03



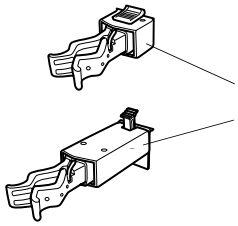
Connection part, (schematic drawing)

Item	Quant./ pack	A/C*	Code no.
Injection valve			
Repair kit INV-907	1	C	18-1109-05
Injection fill port 0.7 mm	1	C	18-1127-66
Buffer valve			
Repair kit IV-908	1	C	18-1109-07
Mixer			
Mixing chambers:			
0.6 ml	1	A	18-1118-90
2 ml	1	A	18-1118-91
5 ml	1	A	18-1118-92
12 ml	1	A	18-1118-93
Fraction collector			
Tube racks, complete with bowl, tube support, holder and guide:			
12 mm	1	A	19-8684-03
18 mm	1	A	18-3050-03
Tube support	1	A	18-3054-02
Tube holder and guide:			
12 mm	1	A	19-7242-02
18 mm	1	A	19-8689-02
Eppendorf tube holder for 12 mm rack	100	A	18-8522-01
Flow diversion valve, FV-903 incl. mounting bracket	1	A	18-1114-50
Tubing holder	1	A	18-6464-01
Drive sleeve	5	C	19-6067-02
Sample loops			
10 µl	1	C	18-1120-39
100 µl	1	C	18-1113-98
500 µl	1	C	18-1113-99
1 ml	1	C	18-1114-01
2 ml	1	C	18-1114-02
5 ml	1	C	18-1140-53
Superloop 10 ml, 50 ml			
Superloop 10 ml, complete	1	A	18-1113-81
Superloop 50 ml, complete	1	A	18-1113-82
Inner end piece	1	A	19-7846-01
Outer end piece	1	A	19-5167-01
O-ring, inner end piece	5	C	19-7595-01
O-ring, movable seal	2	C	18-1104-97

	Item	Quant./ pack	A/C*	Code no.
	Movable seal	1	A	19-7845-01
	Protective jacket (50 ml)	1	A	19-7849-01
	Glass tube with thread and groove (10 ml)	1	A	19-7593-01
	Glass tube with thread and groove (50 ml)	1	A	19-5165-01
	Tubing kit for Superloop (10 ml)	1	A	18-1113-83
	Tubing kit for Superloop (50 ml)	1	A	18-1113-84
	Superloop 150 ml			
	Superloop 150 ml, complete	1	A	18-1023-85
	Movable seal	1	A	18-1029-58
	Inner end piece	1	A	18-1029-59
	O-ring, inner end piece	2	C	18-1029-60
	O-ring, movable seal	1	C	18-1134-49
	Cables			
	Mains cable, 120 V	1	A	19-2447-01
	Mains cable, 240 V	1	A	19-2448-01
	Mains distribution lead 0.3 m	1	A	18-1119-05
	Signal cable, ÄKTAprime plus	1	A	18-1141-35
	Connectors and unions			
	Tubing connector, inlet nut for o.d. 3/16", PEEK	10	A	18-1112-49
	Ferrule, for 3/16" o.d. tubing, PEEK	10	A	18-1112-48
	Tubing connector, inlet nut for o.d. 1/8", PEEK	10	A	18-1121-17
	Ferrule, for 1/8" o.d. tubing, PEEK	10	A	18-1121-18
	Union, 1/16" female/M6 male, PEEK	6	A	18-1112-57
	Union, luer female/1/16" male, PEEK	2	A	18-1112-51
	Union, M6 female/1/16" male, PEEK	8	A	18-1112-58
	Union, 5/16" female/M6 male, PEEK	3	A	18-1127-76
	Union, 1/16" male/1/16" male, for 1/16" o.d. tubing, PEEK	10	A	18-1120-92
	Union, 1/16" female/1/16" female, for 1/16" o.d. tubing, titanium	1	A	18-3855-01



Item	Quant./ pack	A/C*	Code no.
Fingertight connector 1/16", for PEEK tubing o.d. 1/16"	10	A	18-1112-55
Stop plug, 1/16", PEEK	5	A	18-1112-52
Stop plug, 5/16", PEEK	5	A	18-1112-50
Tubing			
Teflon tubing, i.d. 2.9 mm, o.d. 3/16" (IN)	3 m	A	18-1112-47
PEEK tubing, i.d. 0.50 mm, o.d. 1/16"	2 m	A	18-1113-68
PEEK tubing, i.d. 0.75 mm, o.d. 1/16" (G)	2 m	A	18-1112-53
PEEK tubing, i.d. 1.0 mm, o.d. 1/16" (W)	2 m	A	18-1115-83
Sample tubing kit	1	A	18-1115-77
Purge kit	1	A	18-1153-28
Miscellaneous			
Inlet filter assembly	2	A	18-1113-15
Inlet filter set	10	C	18-1114-42
On-line filter	1	A	18-1112-44
On-line filter kit	10	C	18-1027-11
Screw lid GL45, incl. cap membrane	1	A	11-0004-10
Flow restrictor, FR-902	1	A	18-1121-35
Flow restrictor, FR-904	1	A	18-1119-63
Cramp, for column holder, ÄKTAprime plus	1	A	18-1142-71
Column holder, for one column, short	1	A	18-1113-17
Column holder, for one column, long	1	A	18-1126-32
Tubing cutter	1	A	18-1112-46
U-wrench, M6	1	A	19-7481-01
U-wrench, 1/4"	1	A	18-1112-45
Hex wrench, 2.5 mm	1	A	19-4442-01
Chart recorder REC 112, 2 channel	1	A	18-1132-33
Computer, laptop	1	A	18-3535-35



Item	Quant./ pack	A/C*	Code no.
User Documentation			
ÄKTAprime plus User Manual	1		11-0026-44
ÄKTAprime plus Cue Cards	1		11-0027-48
Related literature			
Protein Purification Handbook	1		18-1132-29
Affinity Chromatography, Principles and Methods	1		18-1022-29
Gel Filtration, Principles and Methods	1		18-1022-18
Hydrophobic Interaction Chromatography, Principles and Methods	1		18-1020-90
Ion Exchange Chromatgraphy & Chromatofocusing, Principles and Methods	1		11-0004-21
Antibody Purification Handbook	1		18-1037-46

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